SHOUT
SHARE
IT

3977
Amutlin.
10

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

Editorial Staff

E. G. Conklin—*The University of Pennsylvania.*
Jacques Loeb—*The University of California.*
T. H. Morgan—*Columbia University.*
W. M. Wheeler—*American Museum of Natural History, New York.*
C. O. Whitman—*The University of Chicago.*
E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

Smithsonian Institution
254051
National Museum.

Volume XIII.

WOODS HOLL, MASS.
JUNE TO NOVEMBER, 1907.

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

CONTENTS OF VOLUME XIII.

No. 1. June, 1907

PAGE

O. C. Glaser. *Pathological Amitosis in the Food-ova of Fasciolaria* 1

Bertram G. Smith. *The Life History and Habits of Cryptobranchus allegheniensis* 5

Helen Dean King. *Food as a Factor in the Determination of Sex in Amphibians* 40

No. 2. July, 1907

Kristine Bonnevie. "*Heterotypical*" *Mitosis in Nereis limbata* (*Ehlers*) 57

Roy L. Moodie. *The Sacrum of the Lacertilia* 84

Pauline H. Dederer. *Spermatogenesis in Philosamia cynthia*. 94

No. 3. August, 1907

Max M. Ellis. *The Influence of the Amount of Injury upon the Rate and Amount of Regeneration in Mancasellus macrourus* (*Garman*) 107

J. F. McClendon. *The Spermatogenesis of Pandarus sinuatus Say* 114

J. Thomas Patterson. *The Order of Appearance of the Anterior Somites in the Chick* 121

Adele M. Fielde. *Suggested Explanations of Certain Phenomena in the Lives of Ants; with a Method of Traciug Ants to their Respective Communities* 134

C. M. Child. *Studies on the Relation between Amitosis and Mitosis, III.* 138

Harold Heath. *The Longevity of Members of the Different Castes of Termopsis angusticollis* 161

No. 4. September, 1907

C. M. Child. *Studies on the Relation between Amitosis and Mitosis, IV.–VI.* 165

William M. Wheeler. *On Certain Modified Hairs Peculiar to the Ants of Arid Regions* 185

Theodore C. Burnett. *Can Sea Water Maintain the Beat of the Heart of Fresh Water Animals?* 203

Edwin Chapin Starks. *On the Relationship of the Fishes of the Family Siganidæ* 211

Gary N. Calkins. *The Fertilization of Amœba proteus* 219

No. 5. October, 1907

PAGE

Mary Blount. *The Early Development of the Pigeon's Egg, with Especial Reference to the Supernumerary Sperm Nuclei, the Periblast and the Germ Wall* 231

J. Thomas Patterson. *On Gastrulation and the Origin of the Primitive Streak in the Pigeon's Egg.—Preliminary Notice.* 251

T. H. Morgan and C. R. Stockard. *The Effects of Salts and Sugar Solutions on the Development of the Frog's Egg...* 272

L. H. Gregory. *The Segmental Organ of Podarke obscura...* 280

C. R. Stockard. *A Peculiar Legless Sheep* 288

No. 6. November, 1907

D. D. Whitney. *Artificial Removal of the Green Bodies of Hydra viridis* 291

N. Yatsu. *A Note on the Adaptive Significance of the Spermhead in Cerebratulus* 300

Thomas H. Montgomery, Jr. *On Parthenogenesis in Spiders..* 302

S. J. Holmes. *Rhythmical Activity in Infusoria* 306

D. H. Tennent. *Further Studies on the Parthenogenetic Development of the Starfish Egg* 309

Fernandus Payne. *The Reactions of the Blind Fish, Amblyopsis spelæus, to Light* 317

S. W. Williston. *The Antennæ of Diptera; A Study in Phylogeny* 324

C. H. Turner. *Do Ants Form Practical Judgments?* 333

Julian Mast Wolfsohn. *The Causation of Maturation in the Eggs of Limpets by Chemical Means* 345

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

Vol. XIII | June, 1907 | No. 1

CONTENTS

		Page
Glaser, O. C.	*Pathological Amitosis in the Food-ova of Fasciolaria*	1
Smith, Bertram G.	*The Life History and Habits of Cryptobranchus allegheniensis*	5
King, Helen Dean	*Food as a Factor in the Determination of Sex in Amphibians*	40

Smithsonian Institution
National Museum

Published Monthly by the

MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

Agent for Great Britain	Agent for Germany	Agent for France
WILLIAM WESLEY & SON	R. FRIEDLÄNDER & SOHN	LIBRAIRIE ALBERT SCHULZ
28 Essex Street, Strand *London, W. C.*	*Berlin, N. W.* *Carlstrasse, 11*	*3 Place de la Sorbonne* *Paris, France*

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter,
under Act of Congress of July 16, 1894.

Editorial Staff.

E. G. Conklin—*The University of Pennsylvania.*
Jacques Loeb—*The University of California.*
T. H. Morgan—*Columbia University.*
W. M. Wheeler—*American Museum of Natural History, New York.*
C. O. Whitman—*The University of Chicago.*
E. B. Wilson—*Columbia University.*

Managing Editor.

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Holl, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa.

Vol. XIII. *June, 1907.* *No. 1.*

BIOLOGICAL BULLETIN

PATHOLOGICAL AMITOSIS IN THE FOOD-OVA OF FASCIOLARIA.[1]

O. C. GLASER.

In his paper entitled "Amitosis in the Embryo of *Fasciolaria*" ('04), Professor H. L. Osborn has described the nuclear changes occurring in the food-ova with which the embryos of *Fasciolaria tulipa* gorge themselves at a certain stage of their development ('05). Since the appearance of Child's paper ('07) will no doubt stimulate fresh interest in direct nuclear divisions, I have decided to publish this note on pathological amitosis, particularly as Professor Osborn's description is unsatisfactory. Not only has he conceived an erroneous idea of the structure of the nuclei in question, but he has failed to point out the lesson which they teach, for nuclear divisions which have in common only the property of being non-mitotic, are for that reason not necessarily comparable in other respects.

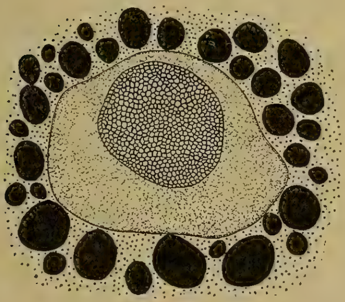


FIG. 1. Unfragmented germinal vesicle of food ovum. The large black bodies represent yolk.

The germinal vesicles of the food-ova, placed excentrically in the eggs, surrounded by a zone of cytoplasm comparatively free from yolk granules, are surprisingly large. The only regions of these vesicles that stain are the enormous nucleoli in which the chromatic material is located between the bubbles of a fine non-staining froth. Outside of each nucleolus, the nuclear material is composed of minute granules which in the preserved specimens studied have the appearance of a more or less definite reticulum suspended in a homogenous ground substance. The vesicles are bounded by a definite membrane outside of which is granular cytoplasm with large yolk spheres. It is quite evident from the legend beneath Fig. 6, p. 875, of Professor Osborn's paper ('04) that he misinterpreted what he saw, for he figures the germinal vesicle alone, and says that it is "The nucleus and the immediately adjacent cytoplasm," mistaking the nucleolus for the nucleus, and the non-staining portion of the vesicle for cytoplasm.

---

[1] Contributions from the Zoölogical Department, University of Michigan, No. 109.

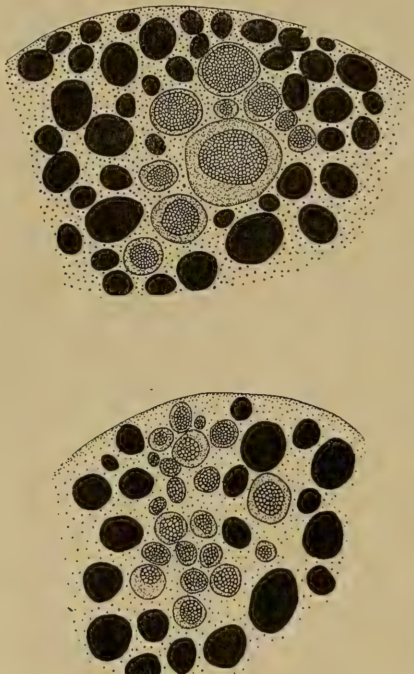


FIG. 2. Fragments of two germinal vesicles. The large black bodies represent yolk.

After the food ova have been ingested a number of days, the germinal vesicles fragment, and the appearance which they present at that time is much as though they had exploded. Instead of finding a single nucleus, one sees numerous fragments, varying greatly in size, and in each case, miniatures of the original germinal vesicle. Each fragment has a central mass, frothy in structure, and staining deeply in the spaces between the bubbles — a piece of the original nucleolus. Surrounding this stained region, is a clear zone of finely granulated nuclear substance bounded by a definite membrane. In some cases the "nucleoli" of these fragments are irregular, but usually their outlines are oval and smooth.

I have not been able to find the elongated dumb-bell shapes described by Osborn ('04, Fig. 7), and interpreted by him as late stages in amitosis; neither have I succeeded in satisfying myself that such intermediate stages as my material shows, are common transition stages between the unfragmented and the fragmented nuclei for not only are these intermediate stages very rare, but they are unconvincing when found, and the appearance which the fragmented vesicles generally present suggests that the divisions took place quickly, perhaps by explosion. I have, however, in a few cases seen constrictions in several of the fragments as though these were undergoing one or more fissions (Fig. 3).

Besides showing several cases of what may be fission, Fig. 3 illustrates two other degenerative changes which occur when the ova are about to disintegrate. Frequently large vacuoles are found inside of the "nucleoli," in some instances apparently bursting outward into the nucleoplasm, somewhat as the vacuoles in the external kidneys do ('05). These vacuoles in the "nucleoli" seem to originate from the bubbles of the chromatic froth but of this I am not certain. The other degenerative change is the presence in a few instances of small densely staining masses to which Osborn ('04) has drawn attention, and which he compared to the "spore-like bodies" described by Herrick ('92) in the degenerating nuclei of the yolk cells in the egg-nauplius of Alpheus. These bodies I believe are condensations of the chromatin, and they may be found lying either in the "nucleolus," or outside it in the nucleoplasm.

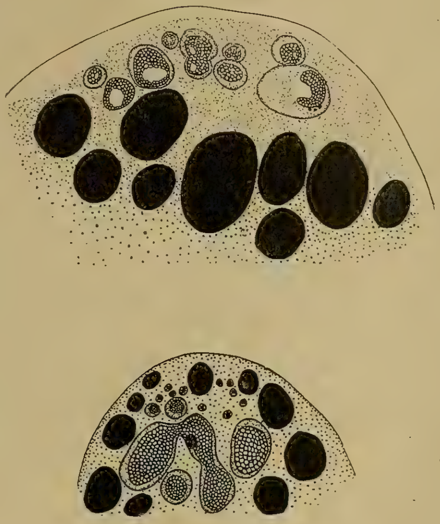


FIG. 3. Fragments of two germinal vesicles showing "spore-like bodies" in upper right hand fragment; vacuoles in the "nucleoli"; and what may be cases of fission. The large black bodies represent yolk.

In concluding his section on the nuclear phemomena in the food ova, Professor Osborn says: "The cells in which the nuclei have undergone these changes are on the road to complete breakdown, and these changes are the last events in their lives. The process is a futile attempt at segmentation where normally we should find mitosis, but in this case the cell having the impulse to divide, but being powerless to do so by mitosis, falls back on the easier mode and does so by amitosis." That the food ova "are on the road to complete breakdown" is unquestionably true, but that the nuclear activities described should be looked upon as futile attempts at segmentation, involving the substitution of amitosis for mitosis, seems to me in the highest degree doubtful. The ova of *Fasciolaria* that develop, are fertilized before maturation, and as the food ova are not fertilized ('05) and consequently not matured, attempts at cleavage are hardly to be expected. Among the conditions to which the eggs are subjected, it is conceivable that they might find stimuli to mature without impregnation, but the nuclear phenomena actually observed are so utterly different from any of the other known kinds of nuclear division, that to interpret the process as a futile attempt at either maturation or segmentation, is to blind oneself with metaphor. To include without qualification such phenomena as these under the heading "amitosis," especially if it becomes established, as seems likely, that under natural circumstances, direct nuclear divisions may intervene between mitotitic divisions without wrecking the ability of the cell to have progeny capable of further differentiation, is certainly inexcusable. It may be etymologically correct to say that a nuclear division other than a mitotic one, is amitotic, but to him who has formed an idea of direct nuclear division from its more usual forms, the word "amitosis" would certainly not suggest Fig. 2 of the present paper. It will be necessary in the future to keep separate the normal and the abnormal events in this field, and to distinguish physiological from pathological amitosis.

Zoölogical Laboratory, University of Michigan,
Ann Arbor, February 25, 1907.

REFERENCES.

Brooks, W. K., and Herrick, F. H.
'92 The Embryology of the Macroura. Mem. Nat. Acad. Sci., V.
Child, C. M.
'07 Studies on the Relation between Amitosis and Mitosis. Biol. Bull., XII.
Glaser, O. C.
'05 Ueber den Kannibalismus bei Fasciolaria tulipa, etc. Zeitschr. f. w. Zool., LXXX.
Osborn H. L.
'04 Amitosis in the Embryo of Fasciolaria. Amer. Nat., XXXVIII.

THE LIFE HISTORY AND HABITS OF CRYPTOBRANCHUS ALLEGHENIENSIS.[1]

BERTRAM G. SMITH.

(Contributions from the Zoölogical Laboratory of the University of Michigan, No. 109.)

I. Historical.

In spite of persistent attempts to work out the natural history of the giant salamander, *Cryptobranchus allegheniensis*, the habits, particularly the breeding habits, have remained little known. Very little has been learned about the development of the eggs, and the larvæ have not been described.

The first account is that of Townsend ('82) and consists of brief notes on the behavior and feeding habits, with a general description of some eggs deposited in August. McGregor ('97) described very briefly an embryo 16 mm. in length. Reese ('03) discussed principally the size, coloration, movements and feeding habits of the adults, and recorded his persistent attempts to obtain embryological material. In a later paper ('04), he gave the first accurate description of the unfertilized eggs.

Recently ('06[2]) I published a detailed description of the eggs and spermatozoa, and an account of the early stages of the development, with some incomplete observations on the breeding habits. The material for this paper was secured during the fall of 1905.

During the months of August and September, 1906, in northwestern Pennsylvania, I devoted my entire time to the study of *Cryptobranchus*, with the object of securing a knowledge of its habits, and material for the study of its development. The purpose of the present paper is to record the results of this work so far as they contribute to a general account of the natural history of the animal.

---

[1] The writer is indebted to the Elizabeth Thompson Science Fund for Grant No. 130.

II. The Adults.

A. *Habits Not Peculiar to the Breeding Season.*

Habitat. — *Cryptobranchus* was found most abundantly in a large creek tributary to the Allegheny River, and the most favorable locality extended from its confluence with the Allegheny five or six miles up the stream.

The stream has a rather rapid descent, and a gravelly or rocky bottom. Shallow and rocky rapids make up the greater portion of its course, alternating with areas of deeper and more quiet water. It is in the former situations (see Fig. 1) that *Cryptobranchus* abounds, lying concealed for the greater part of the time in crevices or caverns under large rocks in the stream bed. It is seldom that more than one individual is found under a rock, hence its life is in general a solitary one.

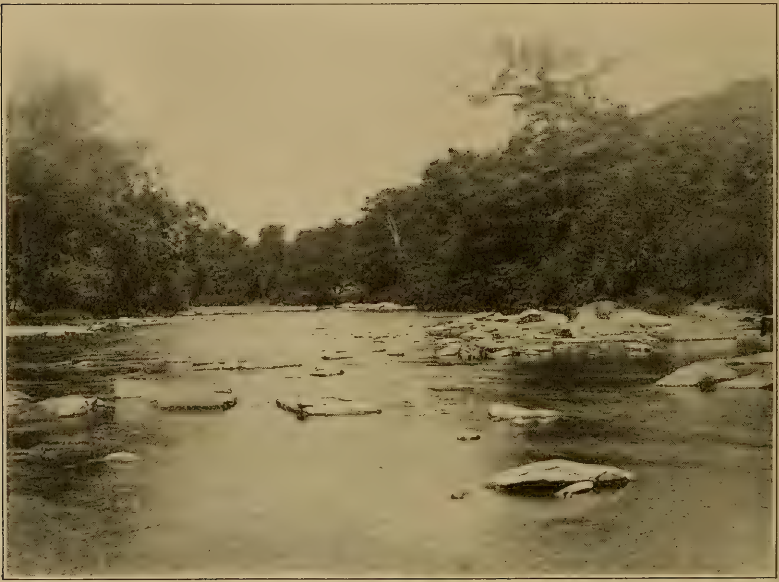


Fig. 1. Typical habitat of *Cryptobranchus alleghéniensis.*

So far as my observations extend, *Cryptobranchus* comes forth but seldom in the day time, except during the breeding season. At night they venture abroad, perhaps in search of food; and fishermen who have speared by torchlight tell marvellous tales of the number of these creatures they have seen, usually lying quiet on the bottom when observed. According to Townsend ('82), in the early summer, when the water is clear, hellbenders are often seen on the bottom in considerable numbers ; in August he found them only under rocks. Presumably his observations were made by daylight.

The cavity or cavern used as a dwelling-place has the rock for its roof and the gravelly bed of the stream for its floor. In perhaps the majority of cases, ready-made caverns are chosen as homes, and these are reached by a natural opening. But the cavity often bears evidence of having been in part hollowed out by the animal, and is sometimes reached by a single burrow-like entrance, with a little heap of freshly excavated gravel at its mouth, on the downstream side of the rock. I have occasionally seen the front limbs used to scratch and push away sand and gravel, while the animal was forcing its way under a rock ; but the burrowing habit is only slightly developed. It is interesting to compare this beginning of the burrowing habit in *Cryptobranchus* with its marked development in the closely related but more terrestrial *Amphiuma*.

There is a striking similarity between the habitat of the American *Cryptobranchus* and that of the giant salamander of Japan (*C. japonicus* v. d. Hoeven, or *Megalobatrachus maximus* Schlegel), as described by Ishikawa ('04) ; but in the case of the latter the burrowing habit appears to be better developed.

Cryptobranchus does not thrive except in cool and shallow running water. When a specimen is placed in a tank of quiet water, it soon shows great uneasiness, swimming restlessly about as if seeking means of escape, and coming frequently to the surface for air. In a short time, as stated by Reese ('03), the stomach contents are regurgitated. So long as kept in such an unfavorable situation, it refuses food.

Size. — Although during the season I handled more than a hundred specimens, the largest adults of both sexes measured only 53 cm. in length. Townsend ('82) records the capture of some specimens 22 inches (56 cm.) long. The longest specimens obtained by Reese ('04) measured 55 cm. By far the greater number of specimens captured by me were much smaller than the extreme size given; specimens of about 35–40 cm. were apparently most numerous. The smallest sexually mature males measured about 33 cm.; females 35 cm.

Form. — The general form of the body (see Fig. 2) is such as to adapt it to bottom life, and to shallow crevices under rocks. The flattened head is wedge-shaped in a horizontal plane, enabling the animal to force itself into very shallow crevices. The fore limbs are adapted for ordinary locomotion, for climbing over rocks, and for use to a slight extent in burrowing.

As compared with the young, the adult is distinguished by the general looseness and wrinkling of the skin at the sides of the body, forming folds which become more prominent in adult life, and by the flaps of skin on the posterior sides of the limbs. During locomotion these folds and flaps undulate in the water, contributing to the uncouth appearance of the animal; they hang limp when the living specimen is taken out of the water, but become stiffened in preserved specimens.

Coloration. — Young adults vary but little in color or color pattern. The ground color is a dull brown, with conspicuous black spots and less conspicuous yellow spots, irregular in form and distribution, scattered over the dorsal and lateral surfaces. Since the coloration of the young adult is practically the same as that of immature specimens from 16 cm. upward, Fig. 14, from a specimen 26 cm. long, will serve to represent this stage, in which the spots are more conspicuous than in the young larvæ or the more mature adults. In older specimens (see Figs. 2–4) the general color effect may vary in two ways: it may become either greenish brown, or decidedly reddish brown. As stated by Reese ('05), these variations in color occur in both sexes.

The coloration of the dorsal surface is protective, closely resembling the gravelly bottom on which *Cryptobranchus* lives. The spots contribute largely to this general effect. When the stone under which a hellbender lies is overturned, the animal sometimes remains perfectly motionless, as if instinctively relying upon its coloration for protection. On account of its size, and the possession of various means of defense, it is probable that *Crypotobranchus* has few enemies, and its coloration is of value to it principally as a means of concealment while lying in wait for prey. The head, especially, resembles a flat stone covered with silt; when the animal is lying in a favorite position, under a rock with only the head exposed, it is extremely difficult to recognize it.

Locomotion. — The ordinary method of locomotion is by crawling slowly along the bottom. The limbs extend laterally and give little support, so that the abdomen lies in contact with the bottom. The order of movement of the limbs is the same as that of a trotting horse; the limbs which move together in the same direction are associated in diagonal pairs (Marey, '79). The pressure of the body on the ground is always diagonal; the body is supported and moved forward by a right fore foot and a left hind foot at the same time the other two limbs swing free from the ground and reach forward. The result is that the limbs on one side of the body are brought nearest together while those on the other side are extended furthest apart. The movement may be illustrated by two persons paddling a canoe, each with a long double-bladed paddle grasped by the middle; let one execute a stroke on the left hand at the same time the other makes the same movement on the right; let this performance be alternated with a similar one in which each person makes a stroke on the side opposite from that previously taken by him.

Cryptobranchus is also a good swimmer. In swimming the body undulates in a horizontal plane, like that of an eel, and the tail shares in this motion; in the most rapid swimming the motion of the tail is very vigorous, and is the principal means of propulsion. The legs, however, are of considerable use in swimming, both to propel and to guide the body and to preserve its equilibrium; during swimming at a moderate rate of speed the legs are the main propelling organs. The legs preserve their usual order of movement during swimming. Swimming is, then, effected by the combined motion of body, limbs and tail, the limbs playing a more important part in propulsion than the lateral fins of most fishes. Hence the swimming movements, as compared with those of fishes, present an advance toward those of most of the higher animals, which swim by the use of the limbs. *Cryptobranchus* often swims close to the bottom, so that locomotion is effected by a combination of crawling and swimming movements.

Breathing Movements. — The process of breathing by means of the lungs has been briefly described by Reese ('03); the following details may be added. In rising for air the anterior end of the body is stretched slowly in an oblique direction upward;

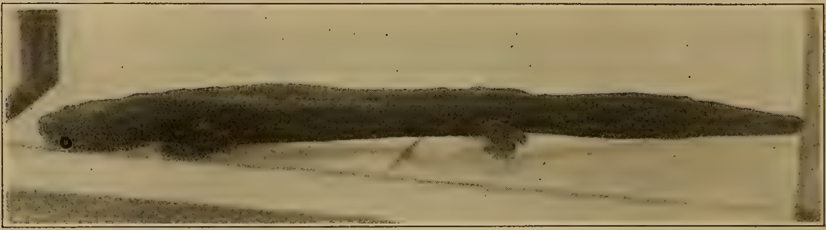


FIG. 2.

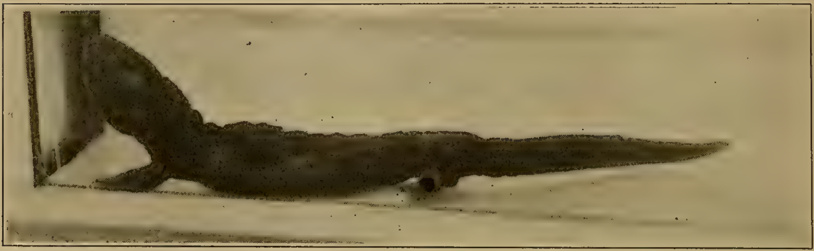


FIG. 3.

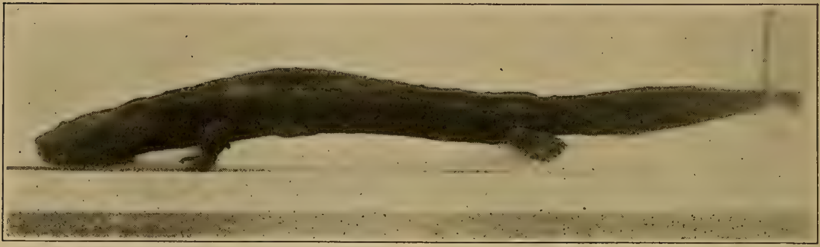


FIG. 4.

FIGS. 2–4. An adult female *Cryptobranchus alleghenienisis*, 53 cm. in length. This specimen, full grown when captured, has been kept for seven years in an aquarium in the Zoölogical Laboratory of the University of Michigan. It is a trifle slender as compared with most newly-captured specimens. The white spots on the back are due to a recent growth of *Saprolegnia*.

FIG. 2. Normal resting position.

FIG. 3. Attitude when about to rise for air.

FIG. 4. Attitude when the lungs are fully inflated.

in shallow water the posterior end often remains under a rock. Fig. 3 shows an early stage of this movement. The air taken in through the nares at the instant the tip of the snout reaches the surface is probably immediately afterwards mixed with respired air expelled from the lungs; then the greater part of the mixed air is forced back into the lungs by a swallowing movement. The surplus air escapes through the mouth or gill slits as the animal sinks to the bottom; but enough air is forced into the lungs to elevate this portion of the body, giving the back an arched appearance (see Fig. 4). A little later, more of this air is expelled in order to enable the animal to resume its normal resting position (Fig. 2), and to prepare it for another inspiration.

According to Whipple ('06), the ypsiloid apparatus (see also Smith, '06 [1]) of many Urodeles subserves a hydrostatic function in changing the angle of inclination of the body in a vertical plane by pressing upon the posterior part of the expanded lungs and forcing the air forward. It is suggested that this may be the case with *Cryptobranchus.* But the animal makes active paddling movements with its forelimbs in rising to the surface for air; moreover when the lungs of a freshly killed specimen are inflated to the limit by means of a blow-pipe, their slender tips do not reach to the ypsiloid apparatus. Hence in the case of *Cryptobranchus* it seems impossible that the apparatus should have a hydrostatic function, excepting the slight effect produced by pressing the abdominal viscera forward.

Specimens in swiftly flowing water in cool weather rarely come to the surface to breathe. I have watched specimens that have remained motionless on the bottom for hours. On one occasion a dozen hellbenders in a covered creek aquarium built of wire netting to allow a constant current of water, were submerged by high water for two days without apparent injury. In these cases cutaneous respiration is probably sufficient; this may be aided by pharyngeal respiration, but in specimens confined in aquaria, having access to air, I have been unable to detect any current of water flowing in at the mouth and out at the gills, such as occurs occasionally in a resting *Necturus.* According to Gage ('91) the oral epithelium of *Cryptobranchus* is stratified and non-ciliated, as is usually the case with Amphibia whose respiration is mostly aquatic. The branchial slit is guarded at its pharyngeal opening by two valve-like flaps, in position and appearance like degenerate gills, which prevent the entrance of water from without. Their respiratory service, owing to their small size, is probably very insignificant.

Feeding Habits. — Examination of stomach contents shows that crayfishes form the principal food of *Cryptobranchus*; fishes are eaten only occasionally. Out of a dozen specimens examined in August, nine were found to contain crayfishes; only three contained fishes. *Cryptobranchus* sometimes takes a hook baited with earthworms, if cast near it. In captivity it will eat almost any small moving animal, or pieces of meat moved along a little to one side of the head. A specimen kept in an aquarium in the Zoölogical Laboratory of the University of Michigan is reported to have eaten frogs on several occasions, and once a toad. One of my newly-captured specimens seized a young *Necturus* about eight inches long, but soon released it. The adult *Cryptobranchus* will eat the eggs or larvæ of its own kind. Specimens kept in a creek aquarium in running water do not refuse food, even immediately after their capture.

Like many other amphibians, *Cryptobranchus* eats its own shed epidermis. The epidermis usually comes off in ragged pieces, giving the animal a tattered and uncouth appearance. The epidermis from the feet comes off like the fingers of a glove. The mouth is sometimes used to aid in the removal of the fragments. The practice of eating the shed epidermis is probably quite common amongst the Urodeles. I have observed a *Triton* (*Diemyctylus*) *viridescens* pulling off and eating the glove-like epidermis from the foot of another specimen. Ritter ('97) states that the shed epidermis of *Triton* (*Diemyctylus*) *torosus* forms an important article of diet for the animal.

Cryptobranchus apparently takes no notice of its prey until the latter approaches within a very few inches of its mouth and a little to one side, then seizes it by a remarkably quick sidewise movement. Perhaps the striking distance corresponds to the distance at which it can see clearly, or it may be the result of experience in catching wary prey. The enormous size of the mouth may compensate for the rather deficient eyesight (see

Reese, '05); it also makes possible the capture of rather large animals as food. No attention is ordinarily paid to pieces of meat unless they are in motion, or attract notice by actual contact. The rocks under which *Cryptobranchus* lurks also afford cover for its prey, hence a food supply may be obtained without leaving shelter.

Although I have handled the animals very often and without caution, I have never been bitten by them. A specimen confined in the Zoölogical Laboratory of the University of Michigan is said to have tried to bite several persons; this happened only after it had become accustomed to its surroundings and to the presence of people about it; probably it was only the usual feeding reaction, which had previously been inhibited by discomfort or fear.

Slime Production. — The surface of the body is at all times slimy enough to be extremely slippery, making the animal difficult to hold except when grasped by the neck. This constant secretion is of value to the animal in protecting the skin from abrasion in gliding over a rough rock, and perhaps also from parasites. But when a hellbender is injured or seized and forcibly held, an abundant flow of sticky, whitish, gelatinous slime, resembling the "milk" from the broken end of a milkweed stem, breaks out over the entire body or sometimes only from the tail. This slime causes a smarting sensation when it comes in contact with the freshly-cut surface of the skin.

Profuse slime production is at least indirectly a result of mechanical stimulation, though probably under the control of the nervous system, and associated with fear.

Reactions to Mechanical Stimuli. — The adult *Cryptobranchus* is strongly thigmotactic, seeking crevices under rocks or an angle of the aquarium in which it is confined — a result not entirely due to a negative reaction to light. It may even force its way under a stone of considerable size where no crevice exists, lifting the stone bodily in the water.

A shock or jar in the water sometimes causes a quick jerking movement, and withdrawal under a convenient cover; but the adult reacts far less readily to a shock than the larva.

When forcibly held, the animal wriggles actively, and sometimes makes a rather loud crackling sound, like that produced by boys who snap the joints of their fingers. The noise seems to come from the articulations of the vertebræ.

When tightly squeezed about the neck, the hellbender sometimes utters a shrill cry, perhaps due to the involuntary expulsion of air from the lungs.

Reaction to Light. — As previously stated, *Cryptobranchus* avoids the light. Like its thigmotactic response, this reaction is an important factor in its customary mode of life, since it usually seeks by day the cover of rocks where, concealed from possible enemies, it lies in wait for prey. Specimens confined in the laboratory are nocturnal in their activities, and during the day seek the darkest corner of the aquarium. According to Reese ('06), the tail is much more sensitive to light than the head or the middle part of the body. This may partly account for the favorite position of the animal — lying under a rock with only the fore part of the head exposed.

B. *Breeding Habits.*

Sexual Differences. — The adult male may be recognized (Reese, '04) by the presence of a swollen glandular ring or ridge of tissue about the opening of the cloaca. This is especially prominent in the breeding season, but may be recognized before the breeding season begins.

Females in the breeding season may be distinguished by the swollen appearance of the abdomen. As the egg-laying season approaches the eggs collect in the uteri, and when a ripe female is held in a vertical position with head uppermost, the lower portion of the abdomen sags and bulges out.

Females were found to be quite scarce or perhaps inaccessible as compared with the males. The ratio in those captured was about 1 : 8.

Breeding Season. — As the time of the beginning of the breeding season was not accurately known, on August 11 I examined the reproductive tract of a newly captured male 34 cm. in length. The vasa deferentia were found to be contracted, about 2 mm. in diameter, and almost empty, containing only a very few spermatozoa, and these at the upper end. The spermatozoa were immotile. In the testes very few spermatozoa were mature.

On August 14 a large female was examined. The ovaries contained several hundred eggs almost fully developed, besides many smaller ones. The oviducts were empty.

Observations of this kind were continued throughout the month. On August 24 a female was found in which a few eggs had descended into the oviducts and become surrounded by a gelatinous envelope. This specimen was somewhat exceptional, as in others examined up to September 1 the eggs had not left the ovaries. Evidently there is considerable individual variation in the time when females ripen. On September 1 the first nest of eggs was found; these were in the first and second cleavage stages, hence had probably been deposited about 24 hours before. On September 3 a female was examined in which the eggs, surrounded by their gelatinous envelopes, had all collected in the lower part of the oviduct — the "uterus" — which was much distended, spindle-shaped, about 10 cm. long by 4 cm. in diameter at its widest part; its thin walls had a rich blood supply.

The preparations of the males for the breeding season proceeded at equal pace with those of the females. On August 31, in a specimen 38 cm. long, having the glandular region about the cloacal opening very much swollen, the vas deferens was found to be much distended with milt, and measured about 8 mm. in diameter. From this specimen the seminal fluid could be easily stripped. The spermatozoa were motile; the motion of the shaft is slow as compared with other forms, but that of the undulating membrane is rapid. The posterior third of the testis was darkened and shrunken, and appeared much degenerated; as stated by McGregor ('99), a wave of maturation begins at the posterior end of the testis and passes forward.

Hence the breeding season in this locality may be said to begin about the last of August. Possibly it is influenced by temperature; cool nights, accompanied by a marked decrease in the temperature of the water, set in three or four days previously. During the breeding season the temperature of the water ranged from 14° to 18° C. The egg-laying season lasts about two weeks.

Oviposition. — In a ripe female, the string of eggs in the uterus is aggregated in a much twisted and tangled mass. The egg capsules at the end of the uterus nearest the cloaca, hence those first formed, contain no eggs; likewise those nearest the oviduct proper, hence the last formed, are empty. The egg envelopes at this time are very soft, much wrinkled, contain no water and therefore fit closely about and between the eggs, taking up very little extra space. The number of eggs in a single uterus was counted in one specimen of average size and found to number 220; in another specimen of equal size 225. Those remaining in the ovary were very small (about 1–2 mm. in diameter), hence could not mature until another season. Therefore the number of eggs deposited by a female of average size in a single season must be about 450—probably more in the case of a large female.

The strings of eggs after deposition are usually found twisted together in a tangled mass, corresponding to their condition in the uterus. The process of egg-laying was observed in aquaria in several instances. Egg-laying generally begins slowly, a short string of capsules containing from six to a dozen eggs protruding from the cloaca for many hours before the main mass is deposited; the majority of the eggs are deposited more rapidly, in a constant stream, the process requiring only about five minutes. The newly laid egg capsules take up water slowly, so that for a day or two the envelopes remain flaccid and much wrinkled. They gradually become plump and turgid (see Fig. 5) by osmosis, affording much more efficient protection to the eggs; the folding of the envelope almost entirely disappears. When the eggs are removed from the water, the egg proper looks much larger than it really is, because magnified by the spherical capsule at the bottom of which it lies.

As already recorded (Smith, '06[2]), of the two spawnings of eggs found during the autumn of 1905, one lot of eggs was in the open, the other partly under a rock. The eggs were not searched for under rocks at this time. In the season of 1906, about a dozen nests were found, all in cavities under rocks, and only a few scattering eggs were found in the open. Hence the deposition of eggs under rocks is the normal procedure, and their occurrence in the open quite exceptional. The "nest" of *Cryptobranchus* is simply the ordinary dwelling-place of the animal, used for spawning purposes.

The absence of black pigment in the eggs is probably correlated with the fact that they are laid in darkness. The eggs of *Necturus*, which are also laid under cover, are likewise devoid of pigment.

During the breeding season the water is unusually low. In one case a nest was found at the extreme water's edge, so that the eggs were barely immersed, yet most of them were developing. In this way, by the periodical drying up of the water in swamps, may have originated the terrestrial nesting habits of *Amphiuma*, described by Hay ('88).

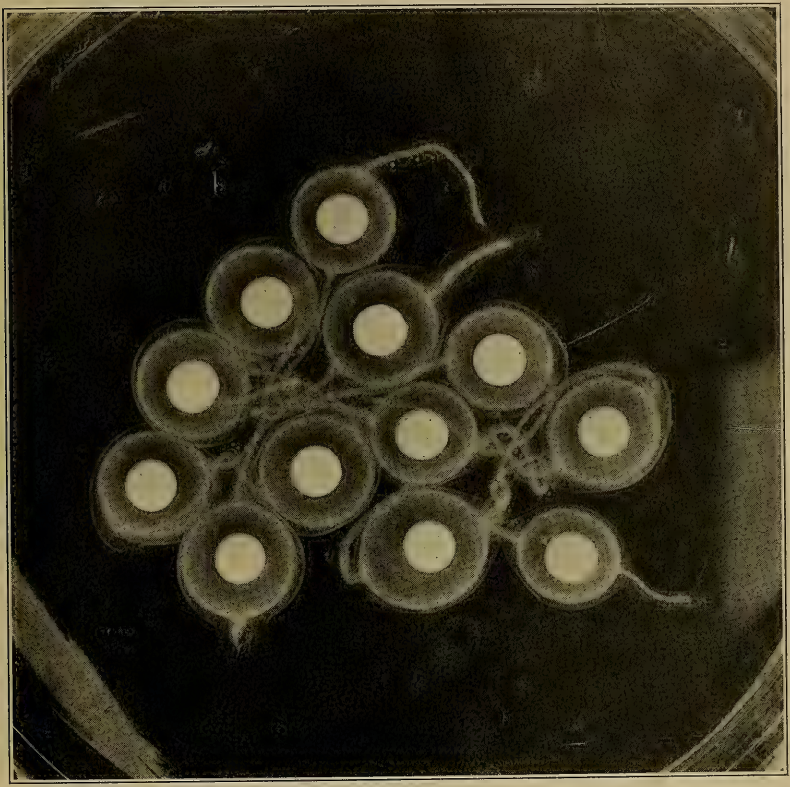


FIG. 5. Unfertilized eggs of *Cryptobranchus alleghenienis*, after two days' immersion in water. Natural size.

Fertilization. — In a previous paper ('06[2]), the conclusion was reached that fertilization in *Cryptobranchus* is internal. The evidence upon which this conclusion was based was afforded by the usual experiment of isolating a ripe female; an hour afterwards a few fertilized eggs were found, although their deposition was not directly observed. It was not known at the time that the female, as well as the male, devours the eggs of its own kind; moreover, the possibility of the regurgitation of the eggs, and their subsequent development, did not occur to me; yet this is undoubtedly what happened. The female *Cryptobranchus* is a voracious eater of the eggs of her own kind; digestion is very slow, and if the animal soon after eating fertilized eggs, is placed in quiet water, the eggs will be regurgitated without injury, and have been observed to continue their development. The number of eggs found on September 6, 1905, was rather large (estimated at about 80) to have been contained in the stomach at one time; but the female was an unusually large specimen, and the eggs must have been devoured before the inflation of their envelopes; hence the feat was quite possible. The evidence for internal fertilization cited above must be regarded as of no value.

The study of the breeding habits was begun with the expectation of finding spermatophores, such as are known to be deposited by *Triton viridescens* (Jordan, '91 and '93) and many other Urodeles (Wiedersheim, '00). Had such spermatophores existed it is quite probable that I should have recognized them through familiarity with spermatophores of *Triton viridescens*, deposited in aquaria, and observations (Smith, '07) on the spermatophores of *Amblystoma* studied in the field; yet, although strings of mucus containing spermatozoa were found floating in the water attached to rocks in localities where *Cryptobranchus* abounds, no spermatophores were found. The search for them was persistent and thorough, both under rocks and in the open, and in creek aquaria where the animals were breeding in large numbers; yet always with the same negative results.

Laboratory studies were conducted to shed light upon the process of fertilization. Eggs taken from ripe females uniformly failed to develop; no spermatozoa were found, either in the cloaca of the female or in eggs taken from the uterus. Females in the act of laying eggs were seized and some of the remaining eggs stripped from the cloaca; no spermatozoa could be found in them and they did not develop. The "opaque body" previously mentioned (Smith, '06[2]), which can be dimly seen in the photograph (Fig. 5), was found to have no essential significance in the process of fertilization, since it is entirely a product of the female, and uniformly present in unfertilized eggs. As stated by McGregor ('99), the female has no seminal receptacle.

Tests were made in order to determine how long living spermatozoa would survive exposure to water. In seminal fluid taken from the vas deferens and thoroughly mixed with water, motion of the sperms, both of the shaft and the filament, continued for about 15 minutes. In drops of seminal fluid obtained by stripping and consequently mixed with the viscid secretion of the cloacal glands, the motion of the sperms continued for four hours after immersion in water.

Artificial fertilization was attempted on several occasions, always with complete success. Eggs for this purpose were secured by killing and cutting open ripe females. Three methods were followed: (*a*) "dry" fertilization — mixing eggs and milt together thoroughly, then immersing in water; (*b*) eggs and milt were placed simultaneously in water, then mixed together; (*c*) eggs were immersed in water for a minute or two, then milt added. All three methods were successful. In every case eggs not artificially fertilized were kept as a check; in no case did these develop. Hence it was proved that the sperms are able to penetrate the egg capsule from without, and that fertilization may take place after both eggs and sperms have been exposed to water.

All the evidence pointed to external fertilization, and it was sought to verify this by actual observation.

With the beginning of the breeding season a marked change took place in the behavior of the animals in their natural environment; they no longer remained secluded in hiding-places under rocks, but came out boldly by day, sometimes congregating in droves of from six to twelve, showing a social instinct quite lacking during the summer. As a rule they roamed restlessly about, poking their noses into crevices under rocks, as if exploring; sometimes, however, they lay quiet in the open. Several times they were observed to pile up in crevices between rocks, two or three lying alongside each other, or two or more at a time try-ing to force their way into the same crevice; this, however, may have been merely incidental to the favorable location. Those confined in aquaria were less shy than formerly, and paid little attention to the presence of an observer; this was true of newly captured specimens as well as of those that had been kept for some time.

Finally, in a large creek aquarium, under conditions made as natural as possible without affording too much cover, the complete process of fertilization was observed at close range in two instances, while various details of the behavior of the animals during spawning were repeatedly observed. The process is essentially as follows:

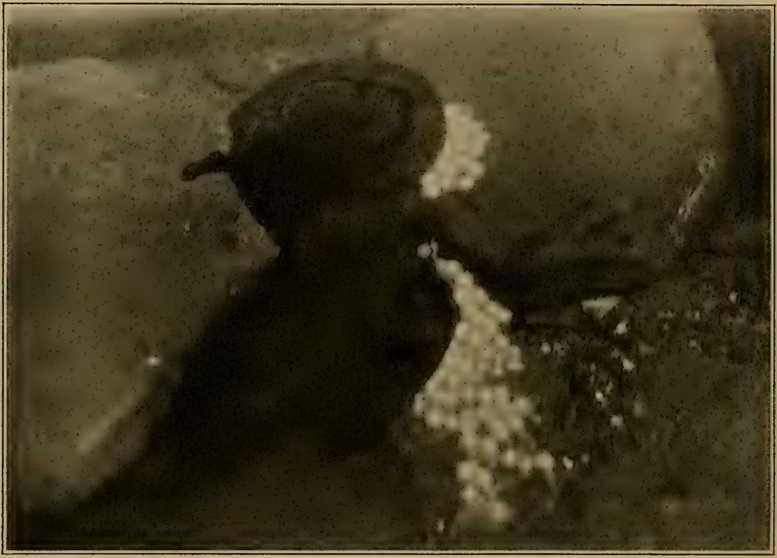


FIG. 6*A*. Photograph from under-exposed negative, showing spawningof *Cryptobranchus allegheniensis*. For explanation see Fig. 6*B*. The photograph was taken with the aid of a water-glass.

The female, with a short string of eggs protruding from her cloaca, crawls restlessly about, dragging the eggs along the bottom; the male, whose attention seems to be attracted by the bright yellow eggs, follows her. Sometimes the female stops, and makes swaying lateral and vertical movements with the posterior part of the body. Finally she crawls partly (in the cases observed) under a rock, and egg-laying begins in earnest. During the remainder of the process of spawning the female lies quite motionless. The male moves to a position alongside and sometimes slightly above the female and overlying the eggs, or sometimes beside them. In this position, he makes swaying lateral and vertical movements of the posterior end of the body, raising and lowering it with his hind legs, much like a male *Triton viridescens* depositing a spermatophore, except that in the case of *Cryptobranchus* the motion is less violent. The movements also resemble those of the female preliminary to spawning. While executing these movements the male extrudes from his cloaca a snowy-white ropy or cloudy mass which consists of seminal fluid mixed with the secretion of the cloacal glands. On one occasion the deposit occurred in ropy chunks about 4 mm. in diameter and 2 or 3 cm. long. The deposit does not necessarily fall directly on the eggs, but sometimes on the ground beside them. Soon after, his own movements or those of other male hellbenders which approach brush the material about until

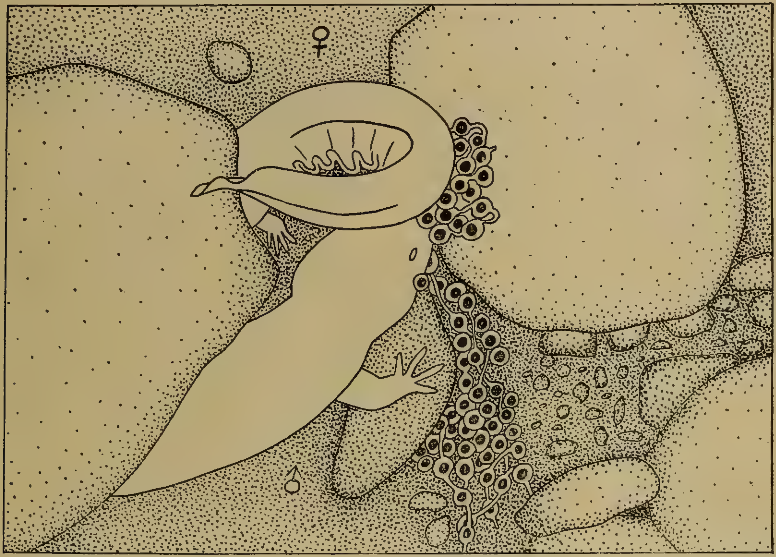


FIG. 6*B*. Drawing explanatory of Fig. 6*A*. The female, in a curved position has her head under a rock; the male, advancing from under the rock, with his foot on a stone, is not yet in position to fertilize the eggs.

it becomes diffused amongst the eggs. It usually happens that while a female is spawning several males are lurking about taking an evident interest in the proceeding. Once, while one male lay amongst the eggs (whether he fertilized them could not be seen) another male about a foot away, and certainly not in contact with the female, was observed to deposit milt. Evidently the excessive number of males is of value to insure fertilization. After fertilizing the eggs, the male sometimes leaves them, but more often remains beside them, or crawls under or amongst them.

Under perfectly natural conditions, spawning and the fertilization of the eggs takes place under large rocks where it cannot be observed; also the male more often remains with the eggs in the normal nesting place.

Photographs of the spawning operations were attempted with poor success (see Fig. 6).

Fertilization is then external as in most fishes, but the eggs are deposited without direct aid from the male. The presence of the string of eggs seems to be sufficient for sex recognition.

With the close of the breeding season, *Cryptobranchus* becomes more shy, avoids the light, and is seldom seen in the open.

Kerbert's account ('04) of the behavior of a pair of captive specimens of *C. japonicus* during spawning will be interesting for comparison. "Schon zu anfang des August 1902 verhielten sich die beiden Tiere anders als gewöhnlich. Wahrend die durchaus trägen, stumpfsinnigen Geschöpfe in der Regel tage- und wochenlang bewegungslos, fast wie tot, auf dem Boden ihres Behälters lagen, nur äusserst langsam nach den ihnen dargebotenen Fischen schnappten, das Licht scheuten und immer die dunkelsten Stellen ihres Behälters aufsuchten, fingen dieselben im August an sich einander zu nähern und gegenseitig zu berühren. Manchmal wurden zitternde und wellenförmige Bewegungen des ganzen Körpers wahrgenommen.

Die Vermutung lag auf der Hand, dass ein Erregungszustand des Nervensystems als Einleitung zur Zeugung eingetreten war. Das Liebesspiel dauerte nur einige Tage. Eine eigentliche Begattung habe ich nicht beobachtet — und auch nicht erwartet." (Kerbert naturally expects fertilization to be accomplished by means of spermatophores). . . .

"Nachdem das grössere, oder — wie ich nacher festzustellen in der Lage war — männliche Tier (1 m. lang) schon seit Anfang September eine unverkennbare Unruhe gezeigt und im Sande am Boden seines Behälters eine deutliche Grube oder Vertiefung gewühlt hatte, fing am 19 September — es war ungefähr sechs Uhr Nachmittags — bei dem kleineren Tiere die Ablage der eigentümlichen Eiermasse an. . . .

Wahrend der Eiablage schwamm das Weibchen in merkbarer Unruhe herum, legte sich aber nach Beendigung dieses Vorganges ganz ruhig hinter den Felsen an der Hinterwand des Behälters. Das grössere Tier war vom Anfang an weit unruhiger und mehr aufgeregt als das Weibchen, schwamm fortwährend durch die von den heftigen Schwimmbewegungen beider Tiere allmählich in die sandige Grube geratene Eiermasse und wehrte die kleinen Fische, Mitbewohner des Behälters, mit geöffnetem Maule von den Eiern ab. Obwohl er sich einige Minuten später scheinbar ruhig bei der Eiermasse hinlegte, war die Erregung des Nervensystems doch offenbar eine so intensive, dass die Haut des Rumpfes und des Schwanzes wellenförmige, zitternde Bewegungen zeigte, ja dass sogar eine heftige Ejaculation von Sperma erfolgte. Eine schleimige grauweisse Masse machte das Wasser trübe. Bei mikroskopischer Untersuchung stellte sich unverkennbar heraus, dass in dieser schleimigen Masse eine grosse Anzahl von Samenfäden anwesend war. Mit voller Bestimmtheit war also nachgewiesen, dass das grössere Tier wirklich ein Männchen war." No deposit of material in a definite mass to form a spermatophore was observed. Kerbert rejects the idea that the emission of seminal fluid on this occasion indicated external fertilization of the eggs laid at that time, and suggests that the spermatozoa diffused in the water might be taken up into the female cloaca to fertilize the eggs for another season; or that the extrusion of milt at this time has no significance in fertilization, being merely incidental to the excitement of the male on account of the spawning of the female. Ishikawa ('04) also inclines to the belief that fertilization is internal, though without conclusive evidence. In view of the results obtained with *C. allegheniensis* it seems very probable that the observations cited above indicate external fertilization in *C. japonicus*.

Considering the low taxonomic position of *Cryptobranchus* amongst the Urodeles, the occurrence of external fertilization in this form indicates that this is the primitive method for the group; internal fertilization by means of spermatophores, characteristic of so many Urodeles, is a later development.

Nothing conclusive is known concerning the method of fertilization in the Crossopterygian Ganoids, the ancestral stock from which the amphibia are believed to be descended. In the higher Teleostomi, with a few notable exceptions, fertilization is external. In the Dipneusti, which, like the Amphibia, are supposed to be derived from a primitive Crossopterygian form, nothing definite is known concerning the manner of fertilization. In view of the diversity of habits within such groups as the Teleostomi and the Urodela, and the wide gaps between the Urodeles and the nearest living representatives of ancestral groups, it is probable that even were our knowledge of the habits of existing animals complete, a comparison between the Urodeles, the Crossopterygii, and the Dipneusti would have little phylogenetic significance. On the other hand, the occurrence of a simple method of external fertilization as the primitive condition of the Urodeles suggests the possibility of finding within the group stages in the development of the complicated breeding habits, involving internal fertilization by means of spermatophores, characteristic of most of its representatives.

Brooding Habits. — It has been noted previously that the male usually remains with the eggs after fertilizing them. That the male may render efficient protection to the newly-laid eggs is shown by the following incident of September 1, which I quote almost verbatim from my notes.

A large "red" hellbender (afterwards identified as a female) crawled across the stream toward a flat rock submerged in about 10 inches of water. This rock was about 3 feet by 2½ feet, slightly tilted so that the down stream side was a little raised from the bottom. As the female approached this rock, the large flat head of another *Cryptobranchus* (afterwards found to be a male) of unusual size was thrust out from under the down-stream edge of the rock and the newcomer was seized at the side of the head by the jaws of the male. She drew back and was released.

She went away a short distance but repeatedly returned and was repulsed in the same manner. Another small specimen (afterwards found to be a male) joined in the attack, but was also seized and repulsed; once he effected an entrance, whereupon a struggle ensued beneath the rock, as was shown by the water becoming turbid. The intruder withdrew.

The large female returned to the attack. She was seized with a firm grip, which lasted several minutes, during which the combatants rolled over and over, and sometimes drifted with the ventral surfaces uppermost. When released, the female swam away for a rod or more, and did not return.

The large male returned to the rock, but kept his head exposed, as if watching for another attack. Sometimes he held his head erect in an aggressive attitude like an angry snake, although it is possible that this was only a movement preliminary to rising for air. At my approach, he assumed this apparently threatening attitude, contrary to the usual custom in such cases of remaining motionless, or withdrawing under the rock.

All the specimens concerned were captured. The rock was overturned, and beneath it was found a cavity containing a large mass of eggs which were immediately swept down stream by the current. When collected they were found to number nearly 600, in first and second cleavage stages.

Another small male and a small *Necturus* were also found under the rock, but not in the cavity containing the eggs; they were on the side most remote from that occupied by the large male. Presumably their presence had escaped his attention, occupied as he was with his enemies.

The large male had a greatly swollen cloacal region, and his struggles when handled caused milt to exude from the cloacal opening. The stomachs of all the specimens excepting the small male found under the rock (the *Necturus* was not examined) were found to contain eggs; the large male contained 23 eggs, the female 22, and the small male 10. In the case of the female, strings of eggs protruded from the mouth and from the branchial aperture. Since the female was found by examination to be a spent one, and spent females at this date were rare, it is very probable that the eggs were laid by her. Even while I was gathering the scattered eggs, another hellbender appeared on the scene and attempted to devour them.

As previously stated, the eggs were in the first and second cleavage stages, and appeared to represent a single spawning. This was the first nest found during the season, and no other nest was found near it.

In the majority of nests found containing eggs in an early stage of development a male was present, a female never. In one case a male was found in a nest containing embryos two or three weeks old; but there is no certainty that he had remained there continuously during that time, or that the embryos were his own offspring.

The number of eggs found in the stomach of a single *Cryptobranchus* usually ranges from 15 to 25; in a few cases this number was considerably exceeded. The digestive processes of the hellbender are extremely slow, and I have taken undigested eggs from the stomach a week after they were eaten.

So far as observed, only recently laid eggs were eaten by the adults. After eggs have been laid several days they are rendered inconspicuous by a covering of silt.

As previously stated, the number of eggs usually deposited in a single nest is about 450 to 500. Nests found late in the season, with eggs in an advanced stage of development, contained nearly the full number of eggs; probably not more than a tenth part, in most cases, had been eaten.

We have here the beginning of a paternal brooding habit, but only the beginning. A male in making a valiant defense of the nest protects the eggs, to be sure, but at the same time he guards his own food supply. Thus in the case of *Cryptobranchus* the brooding habit may have its origin in the feeding habit. On account of the slowness of his digestive processes, and the short period during which, it appears, the eggs are available as food, the male hellbender alone is unable to eat more than a small portion of the eggs. The habit of defending the eggs during the early stages of their development is presumably, even though some of them are eaten, of advantage to the species; and this guarding habit may be initiated in connection with the feeding instinct.

In objection to this interpretation it might be urged that an animal that had just eaten would not be likely to quarrel over more food; but as a matter of fact the hellbender caught attempting to eat the scattered eggs was already gorged with them.

Another possible interpretation demands consideration. In the case described, the male may have been merely holding the nest while awaiting the coming of another ripe female. The hellbenders driven away were all males with the exception of a spent female; there was no opportunity to observe the reception of a ripe female by a male guarding eggs. Evidently further observations are needed to supply conclusive evidence of the origin of the brooding habit.

The aggressiveness displayed by the male *Cryptobranchus* in defending the nest is the first instance I have observed of pugnacity on the part of a Urodele. In these contests over the possession of the nest, the male doubtless has an advantage over a spent female, since the latter seems to be in a weak condition immediately after spawning.

Concerning the brooding habits of *C. japonicus*, Kerbert ('04) says: "Nach Beendigung der Eiablage legte sich das Weibchen offenbar in grösster Ermattung in eine Ecke des Behälters hin und kümmerte sich um das Gelege gar nicht mehr. Das Männchen hingegen hat seitdem die Eiermasse nicht verlassen—ja sogar *die Brut fortwährend bewacht*. . . . Denn sobald das Weibchen der Eiermasse zu nahe kam, stürzte das Mannchen in sichtbarer Wut auf die Mutter los und vertrieb sie . . . kriecht der Männliche Riesensalamander zwischen den verschiedenen Strängen der Eiermasse hindurch und bleibt dan von der Eiermasse umhüllt liegen, oder er legt sich einfach neben die Eiermasse hin. In beiden Fällen aber hält er, hauptsächlich durch eine pendelartige Bewegung des ganzen Körpers, von Zeit zu Zeit die ganze Eiermasse in Bewegung. Durch diese Bewegung entsteht eine für den Atmungsprozess der Eier und Embryonen höchst wichtige Wasserströmung, während die Lage der Eiermasse hierdurch gleichzeitig fortwährend wechselt."

III. The Embryo.

An abundance of fertilized eggs was collected, but on account of the lack of proper facilities great difficulty was experienced in keeping the material alive long enough to secure a complete series. By far the greater part of the eggs perished from variations in temperature, insufficient aeration, or the attacks of water-mold, and it was only by constant search for new nests that the later stages were obtained.

Segmentation.— The segmentation stages have already been described (Smith, '06^2), and since I expect to make a detailed study of the embryology the subject of a special paper, only a few brief notes on the development of the embryo need be given here.

In artificially fertilized eggs the first cleavage furrow appears about 18 hours after fertilization. Segmentation proceeds with increasing rapidity, varying with the temperature, and in a few days a blastula is formed having a shallow segmentation cavity with a very thin, almost transparent roof. So far as external changes visible to the unaided eye are concerned, development during the latter part of this process is comparatively slow. It is also a critical period in the development of the egg ; more embryos die at this time if exposed to slightly unfavorable conditions than at any other.

Gastrulation commences in from six to ten days, according to temperature, after fertilization. The blastopore is formed much as in the frog's egg. The process is quite rapid after the appearance of the short deep horizontal groove just below the equator; this groove quickly elongates, becomes crescent shaped, and the horns close to form a complete circle in some eggs, while in others of the same lot subjected to the same conditions, the process of gastrulation has just begun. The yolk plug rapidly diminishes in size.

Origin of the Nervous System.—The broad but shallow neural groove appears at about the time the circle of the beginning blastopore is nearly complete—a day or two after gastrulation commences. A few hours later the neural folds appear as a sharply-defined horse-shoe shaped ridge about the upper end of the neural groove ; the arms extend downward and closer together until they reach the dorsal lip of the blastopore, which by this time has narrowed to a very small circle. The development of the neural folds proceeds rapidly until their closure a day or two after their first appearance.

Eighteenth Day Embryos. — In from two to three weeks after fertilization the embryos reach the condition represented in Fig. 7. At about this time the rudiments of the external gills appear. A broad horizontal pink band along the left side of the yolk sac indicates the beginning of the vitelline vein. The dorsal surface becomes slightly pigmented, the pigment spots being grouped metamerically (cf. McGregor, '97). The embryos squirm slightly when placed in the fixing fluid.

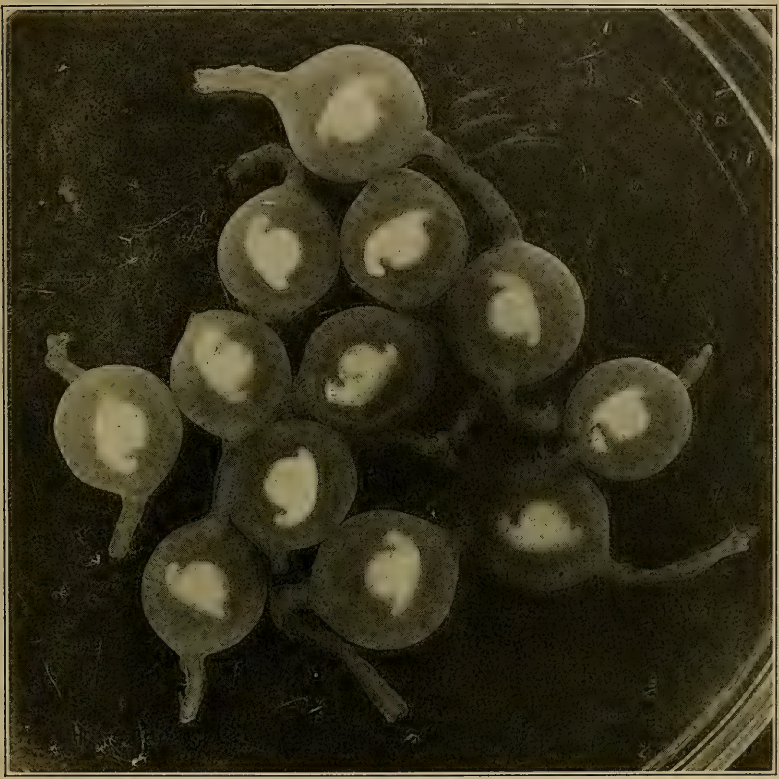


FIG. 7. Embryos of *Cryptobranchus alleghenìensis*, about 18 days old, within egg capsules. Natural size.

Observations in the field were necessarily discontinued at this stage, but living material was transported to the Zoölogical Laboratory of the University of Michigan. The temperature during transportation was regulated with ice. After their arrival at the laboratory the embryos were kept in filtered water aerated with an aspirator, in dishes partly immersed in cool running water. The loss both during and after transportation was very slight.

Four Weeks' Embryos. — Embryos a month old have attained a length of about 20 mm. The eyes are prominent and pigmented ; the entire dorsal side of the body is well covered with black pigment, though the pigment is far from being as dense as in an *Amblystoma* or a frog at a corresponding stage of development. Lateral line sense organs can be distinguished. The three pairs of external gills are well fringed and pink with blood ; the blood corpuscles are large and may be easily observed with a hand lens. The vitelline vein, now very prominent, has shifted to a position along the lower part of the left side of the yolk sac. A conspicuous red spot marks the position of the heart.

Spontaneous movements (exercise movements) now occur. These consist of jerking the head from side to side ; wriggling ; reversal of the laterally curved position of the body by turning over ; swimming movements in which the embryo butts against the envelope and subsides ; swimming in a circle. Embryos removed from the capsule at this stage make practically the same movements ; they are unable to progress in a straight line and incapable of prolonged swimming movements.

Hatching. — Embryos collected when about three weeks old and kept in the laboratory at a temperature averaging about 13° C., began to hatch on October 17, about six weeks after the eggs were probably laid. Nearly all were hatched by October 24. At this time the average length was about 25 mm. The appearance of the embryos just previous to the hatching is shown by Fig. 8.

Before the hatching period, the envelopes become much softened and considerably enlarged by the absorption of water, making room for the growing embryo. The latter sometimes escapes by pushing its way through the envelope, leaving a small round hole ; sometimes apparently by tearing or bursting the envelope by means of its wriggling movements.

In its natural environment the hatching of the embryo is perhaps delayed a little by the slightly lower temperature, but the climatic conditions are such that it seems at least certain that hatching occurs at some time during the fall.

According to Kerbert ('04), the eggs of *C. japonicus*, kept at an average temperature of 13° C., hatched in from 52 to 68 days, and at the time of hatching the embryos were 30 mm. long. Since the extreme length of the adult *C. japonicus* is 159 cm. (Gadow, '01) as compared with 56 cm. for *C. alleghemiensis*, there is far from being a corresponding difference in the size of the embryos at the time of hatching.

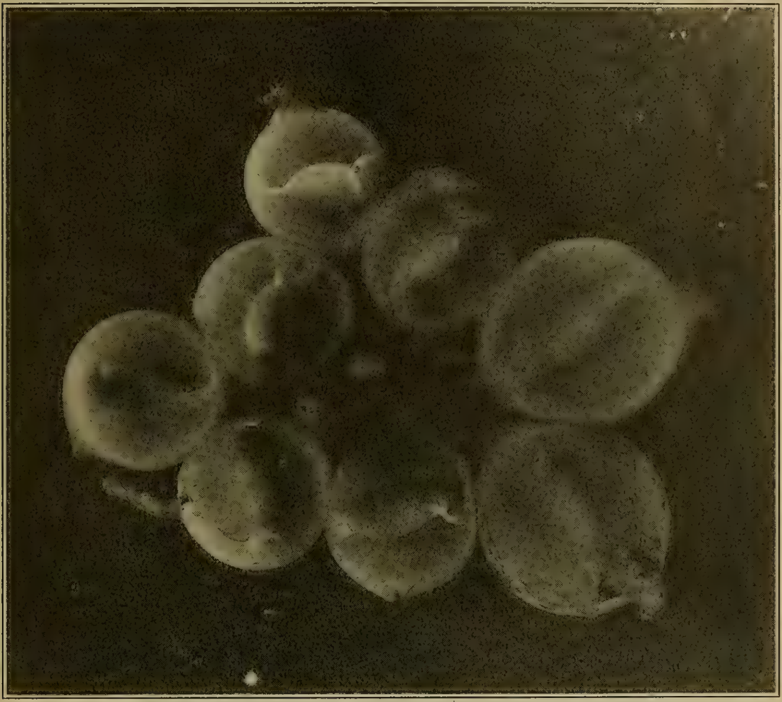


FIG. 8. Embryos of *Cryptobranchus alleghemiensis*, ready to hatch, about six weeks old. Natural size.

IV. The Larva.

A. *Early Stages Studied in the Laboratory.*

The Newly Hatched Larva. — At the time of hatching the larva (see Fig. 9) retains a large yolk sac. The dorsal surface is well pigmented, but the larva is pale as compared with amphibians that live exposed to the light. The ventral surface is lacking in pigment, leaving the abdominal region yellow from the presence of yolk and the throat region white and nearly transparent. The heart can be readily observed without dissection. The vitelline

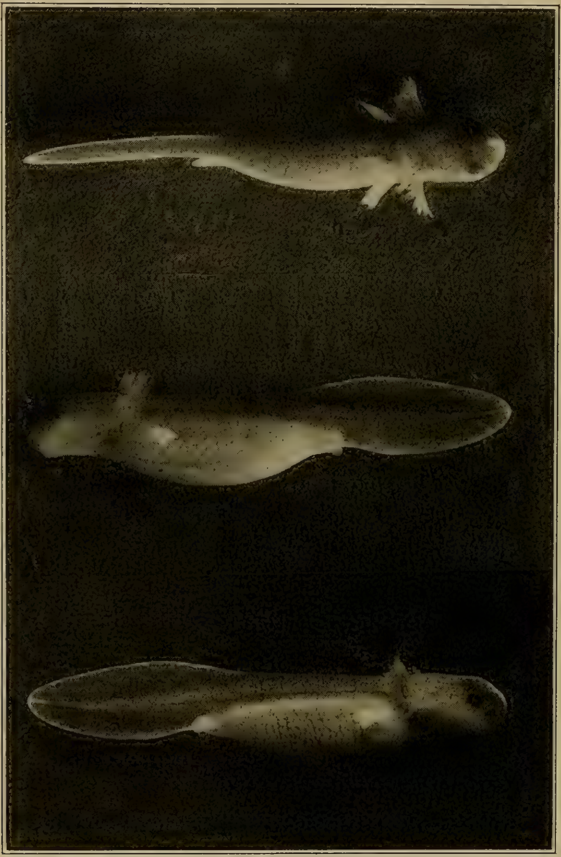


Fig. 9. Newly hatched larva of *Cryptobranchus allegheniensis*, 25 mm. long, photographed while living. × 2½.

vein is very prominent. The anterior limb rudiment is provided with two toes. The somites are plainly visible in most specimens, but do not show in the figure. The mouth opening is large and ventrally situated, and the mouth cavity is well developed. The eyes are more prominent than in the adult. The dorsal and lateral surfaces, especially of the head, are thickly studded with small round white spots, presumably sense organs and mucus glands.

On account of the large yolk sac, the larva habitually lies on its side, turning occasionally from one side to the other. When the larva is placed upon its back, the righting reaction, aided as it is by the ballast of yolk, is very quick. The newly hatched larva is able to swim rapidly in a straight line for a short distance, using the tail as a propeller.

The larvæ avoid the light and are positively rheotactic. Under natural conditions, the result of these two modes of behavior probably is that the larvæ remain in the nest.

Aeration of the blood is afforded, not only by the external gills, but by the capillaries which lie close to the surface all over the body. The tail may be of especial importance in respiration, for here, as in the external gills, the capillary network lies in close proximity to the water on both sides.

At the time of hatching, patches of cilia are scattered over practically the entire surface of the body, but are especially numerous on the gills. Their beat is backward, and they create a current of water, subservient to respiration, which is particularly strong in the vicinity of the gills. The presence of cilia on the ectoderm of Amphibian embryos has been remarked by various writers (see Assheton, '96).

The Month-old Larva. — In larvæ about a month after hatching (see Fig. 10), there was noted a marked increase in length, principally due to the development of the tail. The larvæ now measure from 30 to 35 mm. There is also a rapid elongation and development of the front limbs, which now have four toes. The form and position of the front limbs adapt them for use as paddles. The posterior limbs develop more slowly, are relatively short and have but three toes distinctly visible, though in some cases rudiments of the fourth and even the fifth are present. The yolk sac is reduced enough so that the larva no longer lies on one side. The mouth opening is now only slightly ventral. Pigmentation is greatly advanced, and extends to the external gills; when viewed from above the larva is now nearly black. The ventral side remains white and nearly transparent in the throat region and yellow in the region of the abdomen or yolk sac. The heart is still visible, and the course of the blood through it may be readily followed. The vitelline vein, now lying almost in the median line ventrally, shows signs of degeneration.

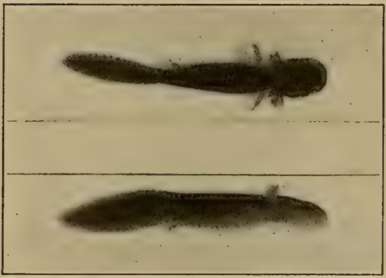


FIG. 10.

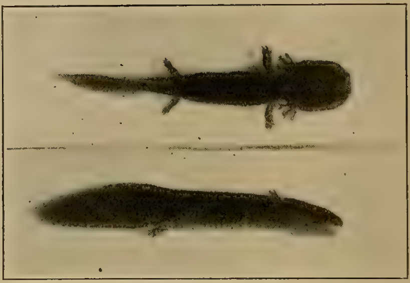


FIG. 11.

FIGS. 10 and 11. Photographs from living material, showing early stages in the development of the larvæ of *Cryptobranchus alleghaniensis*. Natural size.

FIG. 10. Larvæ one month after hatching. Length 35 mm.

FIG. 11. Larva ten weeks after hatching. Length 40 mm.

At this age the larvæ are more active swimmers than the adults. The front limbs assist in starting by a quick simultaneous backward stroke.

Ciliation of the epidermis is now found only on the gills, where a strong eddying current of water is produced. No water current in at the mouth or nares and out through the gill slits could be detected.

At this stage shedding of the cuticle was observed for the first time, and was quite general; the water of the aquaria became cloudy with portions of detached epidermis.

Ten Weeks' Larva. — (See Fig. 11.) There is a slight increase in length since the first month; the larvæ now range from 35–40 mm. The limbs are better developed, possess the full number of toes, and are used in walking in the same manner as in the adults. The limbs are broad and flat, and are used as paddles in swimming at a moderate rate of speed. After fixation the somites show very distinctly.

The yolk sac is greatly reduced, and in some specimens the abdomen presents a shrunken appearance. The vitelline vein is no longer visible. The larvæ seize small pieces of beef offered to them, but do not swallow them.

Six Months' Larva. — Larvæ six months after hatching measure about 40 mm. From the third to the sixth month the increase in length is very slight, but the larva becomes much stouter in structure.

After the fifth month the larvæ not only seize small pieces of raw beef moved along a little to one side of the head, but, to a limited extent, swallow and retain these morsels. The presence of feces in the water indicates that some of the meat is digested.

In larvæ of this age fixed in Tellyesnicky's fluid and preserved in alcohol, the lateral line organs are quite conspicuous; their distribution can readily be made out with the naked eye.

Neither the larvæ of this nor of earlier stages have been observed to come to the surface for air.

The course of larval development described above may be somewhat slower under natural conditions on account of lower temperature. During the period under consideration the temperature of the aquaria ranged from 13° to 9° C., while that of the stream from which the specimens came ranged from 10° to 5° C. It is very probable that the larvæ hibernate in the nest, and perhaps do not emerge until late in the following spring. I have found clusters of 35 mm. *Necturus* larvæ late in August, occupying what was apparently their original nest.

At the time of writing a considerable number of larvæ are being kept alive in the laboratory, and it is hoped that some of them may be reared to the adult condition.

B. *Later Stages Studied in the Field.*

During August of the past summer search was made for larvæ in their natural environment. A few larvæ were found beneath small flat stones in running water only three or four inches deep.

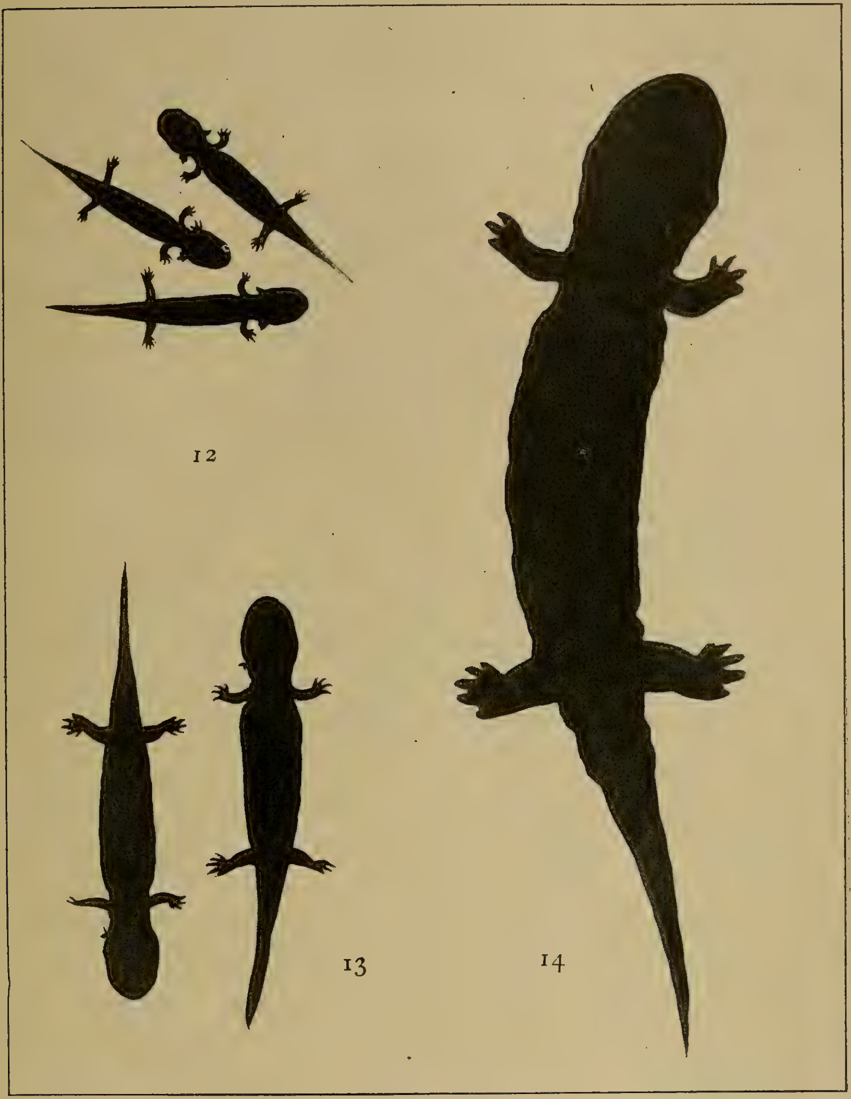
The Year-old Larva.— The smallest specimens obtained (see Fig. 12) were about 7 cm. long. Four specimens were found measuring as follows; 6.4 cm., 6.8 cm., 7.0 cm., 7.3 cm. These were presumably hatched during the preceding autumn. The external gills are retained, though evidently in a somewhat degenerate condition. The legs are well developed. The mouth opening is terminal, not ventral. The tail is proportionally smaller than in specimens ten weeks old; the ratio of length of tail to entire length is 1 : 2.5 in the case of the year-old larva, and 1 : 3 in the ten-weeks' larva. The body is plump as compared with the adult, the somites show distinctly, and the lateral folds are very slightly developed. There is a conspicuous gular fold not present in the adult. The color of the dorsal and lateral surfaces is a very dark brown, almost black, so that the irregular black spots which are present do not show in the photograph. After preservation in formalin the ground color becomes lighter and of a bluish tint, and the black spots show more distinctly. A few scattering inconspicuous yellow spots are also present. The ventral surface is considerably lighter in color.

These larvæ are more active than the adults, and are extremely sensitive to shocks and jars. They were never observed to come to the surface for air.

The Two-year-old Larva. — (See Fig. 13.) Two specimens were found, measuring respectively 12 cm. and 12.3 cm. These differ from the year-old larvæ in the small size of the external gills, in the absence of the gular folds, the slightly greater development of the lateral folds, and in the absence of visible somites, though the lateral folds are metamerically notched. The ground color is a trifle lighter, so that the black spots are more clearly seen; less conspicuous yellow spots are also present. No stages intermediate between these and the 7 cm. larvæ were obtained, and it seems probable that they represent the second year of larval development.

One of these specimens ate, soon after being captured, a large *Corydalis* larva. Another specimen, when placed in quiet water, regurgitated a partly digested 6 cm. larva of its own kind.

As in the younger stages, these specimens were not observed to come to the surface for air; however, the evidence on this point is not conclusive.



FIGS. 12–14. Larval and post-larval stages of *Cryptobranchus alleghenienensis*, ½ linear reduction. From living specimens.

FIG. 12. Larvæ taken August 14, photographed August 17, 1906. Length 6.4 cm., 6.8 cm., and 7.0 cm. respectively.

FIG. 13. Larvæ taken August 14, photographed August 17, 1906. Lengths 12.0 cm. and 12.3 cm. respectively. Reduced external gills are present on both sides.

FIG. 14. Young *Cryptobranchus* 26.7 cm. long.

V. Post-larval Development.

A series of sizes intermediate between the 12 cm. larvæ and the adults was obtained. Six specimens taken during a single week in August measured as follows: 14 cm., 16.2 cm., 18.3 cm., 21 cm., 21.5 cm., 26.7 cm. All these had lost their external gills, but were sexually immature. The lateral folds show increased development throughout the series. The coloration is about the same as that of the young adult; Fig. 14 will serve to show the general appearance of the specimens ranging from 16 to 35 cm., in which the spots are more conspicuous than in either very young or very old specimens.

Sexual maturity is attained with a length of about 34 cm., and probably requires three or four years from the time of the fertilization of the egg.

In conclusion, I take pleasure in acknowledging my indebtedness to Professor Jacob Reighard for training in field work without which it would have been impossible for me to conduct studies of this kind far from the advantages of a laboratory, and to Dr. O. C. Glaser, under whose direction work was continued at the university, for invaluable encouragement and advice.

University of Michigan Zoölogical Laboratory,
Ann Arbor, Michigan.

LITERATURE CITED.

Assheton, Richard.

'96 Notes on the Ciliation of the Ectoderm of the Amphibian Embryo. Quart. Journ. Micros. Sc., Vol. 38, p. 465.

Gadow, Hans.

'01 Amphibia and Reptiles. Vol. VIII. of Cambridge Natural History.

Gage, Simon Henry.

'91 Life-History of the Vermilion-Spotted Newt (Diemyctylus viridescens Raf.). Amer. Nat., Dec., 1891.

Hay, O. P.

'88 Observations on Amphiuma and Its Young. Amer. Nat., Vol. XXII., No. 257.

Ishikawa, C.

'04 Beiträge zur Kentniss des Riesen-Salamanders (Megalobatrachus maximus Schlegel). Proc. Dept. Nat. Hist., Tokyo Imp. Mus., Vol. 1, No. 2.

Jordan, Edwin O.

'91 The Spermatophores of Diemyctylus. Journ. Morphol., Vol. V., No. 2.

'93 The Habits and Development of the Newt (Diemyctylus viridescens). Journ. Morphol., Vol. 8, No. 2.

Kerbert, C.

'04 Zur Fortpflanzung von Megalobatrachus maximus Schlegel. Zool. Anz Bd. XXVII., No. 10.

Marey, E. J.

'79 Animal Mechanism.

McGregor, J. H.

'97 An Embryo of Cryptobranchus. Abstract in Zool. Anz., Bd. 20, p. 29.

McGregor J. H.

'99 The Spermatogenesis of Amphiuma. Journ. Morphol., Vol. XV., Suppl. p. 57.

Reese, Albert M.

'03 The Habits of the Giant Salamander. Popular Science Monthly, April, 1903.

'04 The Sexual Elements of the Giant Salamander, Cryptobranchus allegheniensis. Biol. Bull., Vol. VI. No. 5.

'05 The Enteron and Integument of Cryptobranchus alleghenienis. Trans. Am. Micros. Soc., 27th Annual Meeting, Vol. XXVI.

'06 Observations on the Reactions of Cryptobranchus and Necturus to Light and Heat. Biol. Bull., July, 1906.

Ritter, Wm. E.

'07 Diemyctylus torosus Esch. The Life-History and Habits of the Pacific Coast Newt. Proc. Cal. Acad. Sc., 3d series, Zoölogy, Vol. I., No. 2.

Smith, Bertram G.

'06[1] Note on the Ypsiloid Apparatus of Cryptobranchus. Science, July 6, 1906.

'06[2] Preliminary Report on the Embryology of Cryptobranchus alleghenienis. Biol. Bull., August, 1906.

'07 The Breeding Habits of Amblystoma punctatum Linn. American Naturalist. (In press.)

Townsend, Chas. H.

'82 Habits of the Menopoma. Amer. Nat., February, 1882, p. 139.

Weidersheim, R.

'00 Brutpflege bei niederen Wirbeltieren. Biol. Centralbl., Bd. 20.

Whipple, Inez L.

'06 The Ypsiloid Apparatus of Urodeles. Biol. Bull., May, 1906.

FOOD AS A FACTOR IN THE DETERMINATION OF SEX IN AMPHIBIANS.

HELEN DEAN KING.

Of the many theories that have been advanced regarding the causes that determine whether an animal shall become male or female, the one that nutrition is a dominant factor in sex determination has received much credence. This theory has been supported by the results of numerous feeding experiments made by different investigators on various classes of animals, and also by statistics compiled by Düsing (5) and others with reference to the proportion of males and females in the human race among the offspring of the rich and of the poor.

Three investigators, Born, Yung and Cuénot, have sought by experimental means to ascertain the relation of nutrition to sex determination in amphibians. Born (1), who was the first investigator in this field, found that in a total of 1,272 young *Rana fusca* that had been well nourished during the larval period, 1209 or about 95 per cent. were females, while in 160 young frogs taken from their natural environment only 52 per cent. were females. From the results of these experiments Born concluded that an abundance of food leads to the development of a greater proportion of females. As several investigators have pointed out, Born's results cannot be considered as furnishing conclusive evidence regarding the influence of nutrition on sex determination, for the methods employed in the experiments did not exclude the possibility that other factors than nutrition influenced the results. No account whatever was taken of the many hundreds of tadpoles that died during the course of the investigations and, as Born himself suggests, there is the possibility that the mortality was greater among the males than among the females. In ascertaining the sex of the young frogs, Born examined the gonads *in toto* and did not make use of sections in any case: if the genital organs were large, the individual was classed as a female; if the organs were small, the individual was considered to be a male. Such a method of distinguishing the sexes in young frogs has been found to be unreliable, as at the time of metamorphosis the genital organs are not very well developed and it is often impossible to determine the sex of an individual with any degree of certainty without making a histological examination of the gonads.

The experiments of Yung (13) on *Rana esculenta* were made, primarily, to study the influence of various kinds of food on the development of the tadpoles, but the results seem to furnish positive evidence that the sex of *Rana* is influenced by nutrition. Yung's experiments were carried out with great care, the different lots of eggs being kept under similar external conditions and the food alone differing in the various cases. In considering his results, Yung also failed to take into account the tadpoles that died during the course of the experiments, and he ascertained the sex of only those individuals that underwent metamorphosis. In these experiments the number of females that developed varied from 70 per cent. to 75 per cent. in different cases, the greatest number being found among the lot of frogs that had received only animal food. In a later series of experiments Yung (14) found that in a lot of 100 young frogs that had been fed exclusively on beef, 78 per cent. were females; the number of females was found to be increased to 81 per cent. in a second lot of 100 tadpoles that had been fed on fish; while in a third lot of 100 tadpoles that had received the flesh of frogs as food the number of females was 92 per cent. From an investigation of the sex of 300 young *Rana esculenta* that had developed under natural conditions, Yung concluded that normally the number of females in this species of *Rana* is about 53 per cent. The results of Yung's experiments, therefore, support Born's conclusion that nutrition is a decisive factor in sex determination, an abundance of food leading to the development of a large proportion of females.

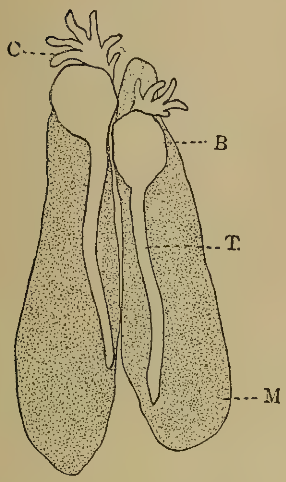
In a recent paper, Cuénot (3) gives the results of a series of feeding experiments which he made on the larvæ of *Rana temporaria* in order to test the conclusion reached by Born and Yung. Cuénot's results do not agree with those obtained by the earlier investigators, as in two lots of frogs that had been well nourished on animal food he found an excess of males; while in another lot of frogs that had been poorly nourished, there was a greater proportion of females. Cuénot states that, as the results of all of the feeding experiments that have been made on *Rana* are contradictory, it is evident that nutrition is not an absolutely dominating factor in sex determination. He believes that there is a strong probability that sex is already determined in the egg at the time of deposition.

As Cuénot used comparatively few individuals in his experiments and as his results do not accord with those obtained by Born and Yung, it is obvious that the question of the influence of nutrition in determining the sex of amphibians is still an open one. It is necessary, therefore, that many more experiments should be carried out along the lines suggested by the work of these investigators.

At the anterior end of the genital organs in the tadpoles of the common American toad, *Bufo lentiginosus*, there is found a small rounded structure, the so-called "Bidder's organ" (Fig. 1, *B*), which is composed apparently of undeveloped ova. The function of this organ is unknown, and whether it is a rudimentary ovary, as many investigators have maintained, has not as yet been satisfactorily determined. This body is found in young tadpoles some time before it is possible to distinguish sex; and it is a permanent organ in the male, disappearing in the female near the end of the second year. If Bidder's organ proves to be a rudimentary ovary, then the adult male toads are in a sense hermaphrodites, although the same cannot be said of the adult female unless the male elements are present in some form that as yet has not been discovered. Because, therefore, of a possible condition of hermaphroditism in the young tadpoles, which might seem to indicate that sex is not already determined at this stage of development, *Bufo lentiginosus* was chosen as more favorable material than any common species of *Rana* for an investigation of the influence of external factors on sex determination. As the tadpoles of *Bufo* are somewhat smaller than those of *Rana* and are easily reared under artificial conditions, they are well adapted for experiments that must, of necessity, extend over a considerable period of time.

The present paper records the results of the first of a series of experiments which have been planned in the hope that it may be possible to show whether external factors such as temperature, nutrition, time of fertilization, etc., have any influence in determining sex in *Bufo*. Recent investigations on other forms have seemed to indicate that sex is not influenced by external conditions, but that it is determined either before or during the fertilization of the egg. If this is indeed the case, experiments such as those that I have in mind will yield only negative results. But if it can be shown for even one form that external factors may be disregarded in considering the question of sex determination, something will have been gained towards an ultimate solution of the problem.

1



2

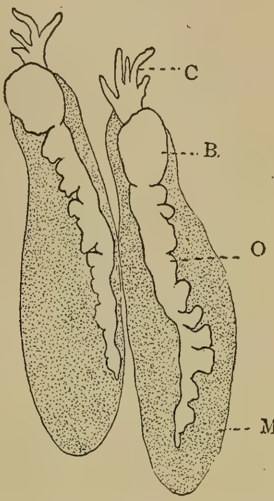


FIG. 1. Camera drawing of the genital organs of a male toad killed soon after matamorphosis. *T*, testis; *B*, Bidder's organ; *C*, corpus adiposum; *M*, kidneys.

FIG. 2. Camera drawing of the genital organs of a female toad killed soon after metamorphosis. *O*, ovary. Other lettering as in Fig. 1.

METHOD.

In endeavoring to ascertain the part played by any one factor in sex determination, it is, of course, absolutely necessary that the influence of all other factors shall be eliminated as far as possible. In making the experiments recorded in the present paper, very great care was taken that all of the individuals being experimented upon were kept under similar external conditions, the only factor that was intentionally varied being that of nutrition whose action it was proposed to study.

Two separate series of experiments were made which, for convenience, will be called Series I., and Series II. The eggs used in Series I., were laid in the laboratory and normally fertilized on the morning of April 13, 1906; while those used in Series II. were laid under similar conditions on April 16, 1906. Both lots of eggs were kept in large aquaria until the tadpoles hatched, and the experiments began in each case five days after the eggs were laid.

Series I. was started with a total of 1,500 individuals; but, as will be explained later, only 1,100 of these can be taken into account in considering the results. Eight hundred individuals were used in the second series of experiments, making a total of 1,900 individuals upon which to base conclusions from the results obtained. Glass dishes of uniform size were used throughout the experiments, each dish containing, in the beginning, 100 tadpoles. The dishes were kept together so that the tadpoles were all under the same conditions of temperature. The water used was "tap" water obtained from an artesian well and used for drinking purposes, so presumably it was free from unicellular organisms. Approximately the same quantity of water was kept in each dish.

Out of the total of 1,900 individuals, only 364, or 19.15 per cent. died before it was possible to ascertain the sex. This comparatively low rate of mortality during the early stages of development I attribute in great part to the fact that the water in the dishes was never allowed to become foul. When the tadpoles were very small the water was changed on alternate days and the dishes carefully cleaned. Later, as the tadpoles became larger, it was necessary to change the water every day. During some very warm weather in June when the tadpoles were beginning to undergo metamorphosis, the water was renewed as often as four or five times daily.

In *Bufo* the genital organs are apparently much better developed at the time of metamorphosis than they are in *Rana*, as in the majority of individuals it is possible to distinguish the males from the females with absolute certainty without making a histological examination of the gonads. The method used to distinguish the sexes in very young toads was as follows: the toad was placed in a flat, shallow dish containing a layer of paraffine which makes an excellent surface for cutting, and the body cavity was then opened under a dissecting lens; the kidneys and the genital organs attached to them were removed by means of small, sharp knives, and subsequently, under a much stronger lens, the gonads were examined *in toto.* Figs. 1 and 2 show the differences between the gonads of the two sexes in young toads that have recently completed metamorphosis. The testes (Fig. 1) are at this time about 2 mm. in length, they are relatively narrow, cylindrical bodies with a smooth outline; the ovaries (Fig. 2), on the contrary, are usually broader than the testes and they have an irregular, jagged outline. Bidder's organ (Figs. 1 and 2, *B*) is very prominent in all individuals at this time; but as it is practically the same size in both sexes, it is of no aid in distinguishing males from females.

In order to ascertain whether the external appearance of the genital organs (as shown in Figs. 1 and 2) is a positive indication of the sex of the individual, 50 young toads were selected of which 25 had gonads approximately like those shown in Fig. 1, and 25 had gonads similar to those in Fig. 2. The gonads were stained *in toto* with hæmatoxylin and sectioned. The histological examination proved conclusively that the external appearance of the gonads can be relied on to indicate the difference in sex, as the sections showed unquestionably that there were 25 males in the one lot and 25 females in the other. At the time of metamorphosis the genital organs are not equally well developed in all individual, however, and occasionally it is impossible to distinguish the sex of a toad without making use of sections.

All of the tadpoles that died during the course of the experiments were fixed in corrosive-acetic (5 per cent. acetic acid) if the hind legs were well developed and the sex ascertained, when possible, by means of sections. A histological examination of the gonads enables one to ascertain the sex of a tadpole some time before the front legs have appeared; for, although the germ-cells may appear similar at this time, the ovary has a central cavity which is not present in the testis. Altogether the gonads of about 600 individuals were examined histologically and in only about 50 cases was it impossible to distinguish one sex from the other.

The methods used in carrying out the experiments and in ascertaining the sex of the individuals have been given in considerable detail in order to indicate the precautions that were taken to avoid the most probable sources of error that might have had an influence on the results.

The Normal Proportion of the Sexes in Bufo lentiginosus.

Cuénot has collected the statistics that have been published regarding the normal proportion of the sexes in various species of *Rana,* and his table shows that the number of females varies from 49 per cent. to 86.8 per cent. in different cases. Pflüger (9) and von Griesheim (7), who have most carefully investigated this subject, find that not only does the proportion of females vary somewhat in lots of frogs taken from different localities, but that there is also a marked difference in the proportion of females in lots of frogs taken from the same locality in different years. The normal proportion of the sexes in *Rana* seems, therefore, to be a variable one depending on the locality and on the year. In the great majority of cases there seems to be a greater number of females than of males, not only among adult frogs but also among the young just after metamorphosis: the excess varies from 1.05 per cent. to 73 per cent. in different cases.

I have not been able to find any statistics regarding the normal proportion of the sexes in other amphibians. Fischer-Sigwart (6) has noticed an excess of males among *Hyla aborea* during the breeding season, and Boulenger (2) has stated that there is an excess of males among the common European toads, *Bufo vulgaris* and *Bufo clamata:* neither investigator gives any statistics in support of his statement. For some years past I have been collecting adult toads during the breeding season and also during the summer months, and I have always found an excess of males in this species. Unfortunately I have kept no records regarding the proportion of the sexes among adults.

In order to determine the relative proportion of the sexes in young toads that have recently completed their metamorphosis, 500 individuals were collected one morning from the bank of the Susquehanna River at Owego, N. Y., during the latter part of June, 1904. The sex of each individual was ascertained by the method described above, it being necessary to make a histological examination of the gonads in only about twenty cases. The result of the investigation is summarized by hundreds in the following table.

TABLE I.

Number of Individuals.	Males.	Females.
100	51	49
100	44	56
100	46	54
100	48	52
100	52	48
500	241	259

Of the total of 500 individuals, 259 or 51.8 per cent. were females, and 241 or 48.2 per cent. were males. In *Bufo* the excess of females among the young seems to be somewhat less than that among young frogs, as according to an investigation made by von Griesheim of the sex of 440 young *Rana fusca*, 280 or 63.7 per cent. were females.

Although in the adult state the female toad is noticeably larger than the male, it is not possible to distinguish the sex of very young toads by their size alone. Two hundred individuals in this group were sorted according to size and it was found that, in many cases, the larger individuals were males. Any variation that may exist in the size of the individuals at the time of metamorphosis can probably be attributed to the difference in the amount of food that the tadpoles were able to obtain.

EXPERIMENTS.

If food is a decisive factor in sex determination, it may be considered to act in one of two ways: either through the quantity of nourishment that it affords the organism; or through its particular chemical nature as a proteid, a hydrocarbon, etc. An abundant nutrition is held by many investigators to lead to the development of an excess of females; while, on the other hand, scarcity of food, according to Schenk (10) and others, tends to produce relatively more males. Yung maintains that nitrogenous food is highly favorable to the development of females; while Schultze (11) states that food of this character has no influence whatever in determining sex.

It was intended, when the experiments began, to test both of the possibilities mentioned above. Among the 300 individuals of Series I., that were poorly nourished the mortality was so great during the first month of the experiment that it was necessary to abandon, for the time, the study of the possible influence of malnutrition on sex determination. The investigations were therefore confined to an attempt to ascertain whether an abundant nutrition or the character of the food received by the larvæ has any influence in determinating sex. The lot of 300 tadpoles which had received little food was therefore discarded, and all of the remaining individuals received an abundance of the particular kind of food whose influence was being investigated.

In Series I., 300 tadpoles (Lot A) were fed exclusively on a meat diet consisting of small pieces of cooked lamb or beef; 300 tadpoles (Lot B) were nourished on a purely vegetable food consisting of a cooked wheat cereal; a third set of 300 individuals (Lot C) received a mixed diet composed of water plants (*Nitella* and *Spirogyra*) and minute organisms on decayed leaves and bits of wood taken from a pond in which toads breed each spring. Lot C presumably received food similar in character to that normally obtained by amphibian larvæ.

According to experiments made by Danilewsky (4), lecithin has a marked influence on the development of frog embryos: tadpoles fed on it show a great increase in size and in weight over control tadpoles that have not received lecithin as food. Danilewsky's experiments were not continued until the tadpoles underwent metamorphosis, and therefore his results do not indicate whether the increase in the size of the tadpoles was due to a more rapid development or whether it was the direct effect of the lecithin in producing abnormally large individuals. As it is conceivable that a more rapid development or an abnormal increase in size might possibly be factors that would influence the sex of an individual, a fourth set of 300 tadpoles (Lot D) in Series I. were fed exclusively on the yolk of hen's egg which, according to Gautier, contains from 8.43 per cent. to 10.72 per cent. of lecithin. No attempt was made to feed tadpoles on lecithin alone, because in experiments which I made several years ago the mortality among tadpoles that were given lecithin as food was exceedingly great; the individuals dying evidently of starvation, as they were never seen to eat any of the lecithin. Owing to an accident, 100 tadpoles fed on the yolk of egg were killed the second week of the experiment. Lot D, therefore, consisted of only 200 individuals, making a total of 1,100 individuals that are to be taken into account in considering the results of the experiments in Series I.

In order to make possible a comparison between the results obtained in Series I. and those from similar experiments on the eggs of a different female, a second series of experiments were made beginning three days later than those in Series I. These experiments were similar in all respects to those in the first series, except that no attempt was made to investigate the possible influence of a scarcity of food in determing sex, and only 200 tadpoles were used in each lot. Series II. therefore consisted of 800 individuals.

Although detailed observations were made on each lot of tadpoles at intervals of about one week, only the record of Series I. for June 7, will be given. This record will serve to show the differences between the individuals of the various lots that can probably be ascribed to the varied character of the food that the tadpoles received.

Lot A. — The tadpoles fed exclusively on meat were noticeably larger than those fed on any other kind of food. The largest individuals measured 27 mm. in length, thus exceeding, by 3–4 mm., the length of a number of tadpoles of about the same age that had been reared under natural conditions; the smallest individuals in this lot were 19 mm. long and were much larger than many of the tadpoles in the other lots. Many of the meat fed tadpoles had very well developed hind legs at this time, but the front legs had not appeared in any individual as yet. It was noticed that these tadpoles were very much blacker than any of the other tadpoles being experimented upon. A meat diet is evidently as favorable to the development of pigment in the toad tadpole as it is in the Mexican axolotl according to the observations of Shufeldt (12). The mortality in this lot was very low, only 18 individuals having died at the time the record was made.

Lot B. — The tadpoles fed on wheat were, as a whole, considerably smaller than those fed on meat, and they were more uniform in size, the greatest number having a length of about 22 mm. By June 7, three individuals in this lot had begun their metamorphosis, and 38 individuals had died.

Lot C. — The smallest and least developed tadpoles were those that were fed on a mixed diet. The greatest extremes in size were also found in this lot, the body length varying from 10–21 mm. in different cases. In many individuals the hind legs were only just visible, and in the largest individuals they were poorly developed as compared with those of the individuals in other lots. The mortality in this lot was very great, 97 individuals having died by June 7.

Lot D. — The great majority of the tadpoles fed on the yolk of egg were intermediate in size between those fed on meat and those that had received a purely vegetable diet, the average length of these tadpoles being 22–24 mm. The individuals in this lot had developed much more rapidly than those in any of the other lots. By the seventh of June, 8 individuals had begun metamorphosis and many more were on the point of doing so. The mortality in this lot also was very great as 63 individuals had died.

The differences between the individuals in the various lots of Series II. were of the same character and as strongly marked as were those in Series I. The death rate in Series II. was practically the same as in Series I.; the fewest deaths occurring among the tadpoles that were fed on meat and on cereal, the greatest number among those that were nourished on a mixed diet and on the yolk of egg.

Although the tadpoles began to undergo their metamorphosis during the first week in June, the experiments were continued until the middle of July as there was a considerable variation in the rate of development among the tadpoles of the same lot. On July 13, all of the tadpoles still living were fixed in corrosive-acetic, as they had reached a stage of development when it would be possible to ascertain the sex of each individual by means of a histological examination of the gonads.

In the corresponding lots of the two series of experiments there was a remarkable uniformity in the rate of development of the individuals. In both series the tadpoles fed on the yolk of egg underwent their metamorphosis much sooner than any of the others, the last one in Series II. completing its metamorphosis on July 11. These tadpoles were only of average size, and none of them ever reached the length attained by many of the tadpoles that were fed on meat. Lecithin, therefore, may cause a more rapid development, but it does not produce individuals of unusual size. The tadpoles fed on meat grew enormously but this increase in size was not accompanied by a more rapid development; on the contrary, the development of these tadpoles seemed to be greatly retarded and some 50 of them had not begun metamorphosis by the middle of July. According to Yung, a purely vegetable diet is insufficient to transform a frog tadpole into a frog. Such a diet does not seem to be equally injurious to toad tadpoles, however, as comparatively few of the individuals that were fed entirely on wheat died during the course of the experiments, and only about 25 of them had not undergone metamorphosis by July 13.

As presumably the individuals that were fed on a mixed diet received the kind of food that is obtained by tadpoles living under natural conditions, it might be expected that these individuals would be larger and stronger than the others and that they would undergo metamorphosis more quickly than those receiving food that is only exceptionally, if ever, obtained by tadpoles in a state of nature. Much to my surprise the development of the individuals in Lot C lagged behind that of the tadpoles in the other lots, and large numbers of them died during the course of the experiments. On July 13, there were at least 100 tadpoles in Lot C that had not yet begun their metamorphosis.

The sex of all of the individuals used in the experiments was ascertained when possible. The results for Series I. are summarized in the following table.

TABLE II.

Character of Food Given.	Total Number of Individuals.	Sex Not Ascertained.	Males	Females.	Per Cent. of Females.	Total Sex Ascertained.
Meat (Lot A).	300	17	146	137	48.40	283
Wheat (Lot B).	300	38	119	143	54.58	262
Mixed food (Lot C).	300	108	103	89	46.35	192
Yolk of egg (Lot D).	200	49	55	96	63.57	151
Total.	1100	212	423	465	52.36	888

Table III. summarizes the results for Series II.

TABLE III.

Character of Food Given.	Total Number of Individuals.	Sex Not Ascertained.	Males.	Females.	Per Cent. of Females.	Total Sex Ascertained.
Meat (Lot A).	200	19	72	109	60.22	181
Wheat (Lot B).	200	16	76	108	58.69	184
Mixed food (Lot C).	200	43	86	71	45.22	157
Yolk of egg (Lot D).	200	74	56	70	55.55	126
Total.	800	152	290	358	55.24	648

The first conclusion that can be drawn from the above tables is that abundant nutrition alone is not a decisive factor in sex determination in *Bufo*, as in three cases (Series I., Lot A, Lot C; Series II., Lot C) more males than females were produced although all of the tadpoles had been well supplied with food during the entire course of the experiments.

As the tables show, the results of the two series of experiments in which the tadpoles were fed exclusively on meat are not in agreement. In Lot A of Series I., only 48.4 per cent. of the individuals in which sex was ascertained were females; while in the corresponding lot in Series II. there were many more females than males (20.44 per cent.). This result does not support Yung's contention that an excess of nitrogenous food leads to the development of a greater proportion of females, and it seems to indicate that food of this character has no influence in determing sex in *Bufo*. Again more rapid growth, as shown in the case of the tadpoles that were fed on the yolk of egg, cannot be considered as favoring the development of one sex any more than the other; for although in both series there was an excess of females in Lot D, this excess varies considerably in the two series (8.02 per cent.)

and is not sufficiently great in either case to warrant the conclusion that sex has been influenced by the rapid development due to the character of the food. The tables show also that a strictly vegetable diet has seemingly no influence on sex determination in *Bufo*. The slight excess of females in Lot B of each series is but little more than that which, according to my investigations, is the normal excess for the species, and it is therefore well within the limits of possible normal variation. In both series the development of the tadpoles that were nourished on a mixed diet (Lot C) was, for some unknown reason, considerably retarded and the individuals that completed metamorphosis were, as a rule, smaller than those of any of the other lots. Both series gave an excess of males in Lot C. This excess, however, is not great enough to justify the assumption that a slow development tends to produce a greater proportion of males, any more than the excess of females among the tadpoles fed on the yolk of egg warrants the conclusion that rapidity of growth favors the development of a greater proportion of females.

The results of these experiments, therefore, seem to show that the character of the food received by the tadpoles is not in itself a decisive factor in determining sex in *Bufo*, although it has much to do with the rate of development and with the size of the individuals.

The results of the experiments as given in Tables I. and II. are summarized in Table IV.

TABLE IV.

Character of Food Given.	Total Number of Individuals.	Sex Not Ascertained.	Males.	Females.	Per Cent. of Females.	Total Sex Ascertained.
Meat.	500	36	218	246	53.01	464
Wheat.	500	54	195	251	56.27	446
Mixed food.	500	151	189	160	45.84	349
Yolk of egg.	400	123	111	166	59.92	277
Total.	1900	364	713	823	53.58	1536

Of the total of 1,536 individuals in which sex was ascertained, 823 or 53.58 per cent. were females. The excess of females, therefore, is but 1.7 per cent. more than the normal excess as ascertained by the examination of the sex of 500 young toads which had developed under natural conditions. The number of females is greatest in the lot of tadpoles fed on the yolk of egg, being 8.1 per cent. above the normal; and it is least in Lot C where it falls 5.9 per cent. below the normal. These figures are, however, well within the limits of possible normal variation for the frog as determined by the investigations of Pflüger and Griesheim, and presumably, therefore they are also within the limits of normal variation in *Bufo.*

It has been suggested by Born, and emphasized by other investigators (Cuénot, Morgan (8)) that the results obtained in feeding experiments may possibly be influenced by the mortality that occurs during the course of the experiments, individuals of one sex dying more readily than those of the other. During the course of my experiments from 30–150 individuals in each lot died before metamorphosis. These individuals, as I have stated, were preserved and the sex ascertained when possible by means of sections. From the records that were made it appears that tadpoles of one sex did not die in greater numbers than those of the other. In the entire number of individuals that were examined the proportion of the sexes was practically the same; in some lots the females died in greater numbers than the males, while in other lots the reverse was the case. These results confirm Pflüger's contention that there is no relation whatever between mortality and sex among tadpoles reared under artificial conditions.

Taking into consideration the entire number of individuals used in the experiments, it is found that in the total of 1,900 tadpoles, 823 or 43.31 per cent. developed into females; 713 or 37.52 per cent. became males; leaving 364 or 19.15 per cent. in which the sex of the individuals was not ascertained. If we assume, for the moment, that all of the individuals belonging to this 19.15 per cent. would have developed into females (although the investigation of the sex of the individuals that died during the course of the experiments does not warrant such an assumption), the number of females would then be increased to 1,187 or 62.47 per cent. of the whole number of individuals; on the other hand, if all of the in dividuals in which the sex was not ascertained had developed into males, then the number of males would be 1,077 or 56.68 per cent. of the whole number of individuals. On neither of these assumptions is the proportion of the sexes in the 1,900 individuals changed sufficiently to justify the conclusion that the nutrition has any influence in the determination of sex in *Bufo*. The results of these experiments, taken as a whole or in part, seem to show that sex is not determined either by the quantity or by the quality of the food that the larvæ receive. This conclusion agrees essentially with that reached by Cuénot from the results of his investigations on frogs, moths and other forms, and by Schultze from his experiments on mice.

LITERATURE.

1. **Born, G.**
'8. Experimentelle Untersuchungen über die Entstehung der Geschlechtsunterschiede. Breslauer Aertzlichen Zeitschr., Bd. III., 1881.

2. **Boulenger, G. A.**
'97 The Tailless Batrachians of Europe. Ray Society, London, 1897.

3. **Cuénot, L.**
'99 Sur la détermination du sexe chez les animaux. Bull. Sci. de la France et de la Belgique, t. XXXII., 1899.

4. **Danilewsky, M. B.**
'95 De l'influence de la lécithine sur la croissance et la multiplication des organismis. Comptes Rendus des Seances de l'Acad. des Sci., t. cxxi., 1895.

5. **Düsing, C.**
'84 Die Regulierung des Geschlechtsverhältnisses bei der Vermehrung der Menschen, Tiere, und Pflanzen. Jan. Zeit. für Naturwiss., Bd. XVII., 1884.

6. **Fischer-Sigwart, H.**
'98 Biologische Beobachtungen an unsern Amphibien. Vierteljahrs. der Naturf. Gesellsch. in Zurich, Bd. XLIII., 1898.

7. **von Griesheim, A.**
'81 Ueber die Zahlenverhältnisse der Geschlechter bei Rana fusca. Arch. für die gesammte Phys., Bd. XXVI., 1881.

8. **Morgan, T. H.**
'07 Experimental Zoölogy. Macmillan Co., N. Y., 1907.

9. **Pflüger, E.**
'82 Ueber die das Geschlecht bestimmenden Ursachen und die Geschlechtsverhältnisse der Frösche. Arch. für die gesammte Phys., Bd. XXIX., 1882.

10. **Schenk, L.**
'02 Meine Methode der Geschlechtsbestimmung. Verh. v. Internat. Zool. Congresses zu Berlin, 1902.

11. **Schultze, O.**
'03 Zur Frage von den geschlechtsbildenden Ursachen. Arch. für mikr. Anat., Bd. LXIII., 1903.

12. **Shufeldt, R. W.**
'85 The Mexican Axolotl and its Susceptibility to Transformations. Science, Vol. VI., 1885.

13. **Yung, E.**
'83 Contributions a l'histoire de l'influence des milieux physico-chimiques sur les etres vivants, II. Arch. de Zool. exper. et. gen., t. I., 1883.

14. **Yung, E.**
'85 De l'influence des Variations du milieu physico-chimique sur le développement des animaux. Arch. des. Sci. phys. et natur., t. XIV., 1885.

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

VOL. XIII JULY, 1907 No. 2

CONTENTS

PAGE

BONNEVIE, KRISTINE. "*Heterotypical*" *Mitosis in Nereis limbata* (*Ehlers*) 57

MOODIE, ROY L. *The Sacrum of the Lacertilia* ... 84

DEDERER, PAULINE H. *Spermatogenesis in Philosamia cynthia* 94

PUBLISHED MONTHLY BY THE

MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY

THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN	AGENT FOR GERMANY	AGENT FOR FRANCE
WILLIAM WESLEY & SON	R. FRIEDLÄNDER & SOHN	LIBRAIRIE ALBERT SCHULZ
28 Essex Street, Strand *London, W. C.*	*Berlin, N. W.* *Carlstrasse, 11*	*3 Place de la Sorbonne* *Paris, France*

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter,
under Act of Congress of July 16, 1894.

Editorial Staff.

E. G. Conklin—*The University of Pennsylvania.*

Jacques Loeb—*The University of California.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*American Museum of Natural History, New York.*

C. O. Whitman—*The University of Chicago.*

E. B. Wilson—*Columbia University.*

Managing Editor.

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Holl, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa.

Vol. XIII. July, 1907. No. 2.

BIOLOGICAL BULLETIN

"HETEROTYPICAL" MITOSIS IN NEREIS LIMBATA (EHLERS).

KRISTINE BONNEVIE.

As the result of investigations on the chromosomes in *Enteroxenos östergreni* I published some years ago (Bonnevie, 1905, 1906) the following conclusions (p. 384, 1906):

"Die Zahlenreduktion der Chromosomen geschieht bei Enteroxenos durch ihre parallele Konjugation in Synapsis. Die dadurch entstandene Doppelheit der Chromosomen geht weder in der ersten noch in der zweiten Reifungsteilung wieder verloren, sondern tritt noch in den Vorkernen deutlich hervor und verschwindet erst im Laufe der folgenden Zellgenerationen mit der völligen Verschmelzung der konjugierten Chromosomen.

"Die konjugierten Chromosomen haben ihre Teilungsfähigkeit behalten; beide Reifungsteilungen sind somit als Aequationsteilungen zu betrachten, deren Bild jedoch durch die Doppelheit und die Grösze der Chromosomen kompliziert wird. Das rasche Aufeinanderfolgen beider Teilungen trägt zu einer Gröszenreduktion der Doppelchromosomen bei; sie werden jedoch erst im Laufe vieler Zellgenerationen auf ihre ursprüngliche Grösze reduziert."

At the time when I first formed this opinion, no clear evidence existed of the corresponding process in other organisms sufficient to make my conclusions seem improbable. But during the time which passed before the publication of my final work a series of papers (Schreiner, 1904, '05; Grégoire, 1904, '05; Montgomery, 1905) appeared, which — though from different points of view — all agreed in claiming for the first maturation mitosis the general significance of a reductional division, separating the chromosomes, which had conjugated at an earlier period.

Opposed to the apparently strong evidence in favor of a reduction division brought together in these papers, as well as that from the valuable investigations of Schreiner (1906*a*) on the maturation process in *Tomopteris* — my results on *Enteroxenos* stood quite isolated. And the reasons which at an earlier period had seemed strong enough to support my view, might now seem inadequate.[1] Even before the appearance of my final paper (1906) I therefore felt the necessity first of reinvestigating the maturation process in *Enteroxenos* and second, in case of the confirmation of my earlier results of finding another object, in which the behavior of the chromosomes might be more easily followed than in this species.

On reinvestigating my old *Enteroxenos*-preparations as well as new material of the same species, I found, that although it might well be possible, even in this species, to select a series of maturation stages showing the "*Tomopteris*-type" (Schreiner, 1906*a*), yet other structures are present in the chromosomes which do not support the assumption of a reduction division.

Besides the great similarity in the general appearance of the two maturation divisions there is a longitudinal split in the chromosomes at the end of this period — a structure which suggests an interrogation as to the assumption of a reduction division, until the existence of this mode of mitosis has been proved for this very species.

On the other hand, however, I willingly admit that *Enteroxenos* is not a favorable object for a decision of these difficult questions — the chromosomes being very much contracted during the metaphase and so small, that the structures in question are often beyond the limit of an objective demonstration. It therefore seemed desirable to extend my investigations to other species with more favorable chromosome relations.

---

[1] (Added on the proof-sheet, June, 1907.) The truth of this sentence was clearly proved through the appearance of the latest paper by A. and K. E. Schreiner (1907) some weeks ago. Their results seem to prove that "the new observations in my paper were not good, while the good ones (if present) were not new." I hope however, through this and my following publications to show that my main results on the maturation divisions in *Enteroxenos* were correct, and that the doubt which they made me feel with regard to the existence of a reduction division in this species was well founded, even if future observations should show that my interpretation of the new facts would have to be modified.

At Columbia University, New York, where I have spent last winter, I have had the best opportunity of doing this; and I want here to express my most sincere thanks to Professor E. B. Wilson for offering me a table in his laboratory, for his generous liberality in giving me free use of his valuable material, and for the lively interest with which he has followed my work.

In this paper I wish to give a preliminary account of my results on *Nereis limbata* Ehlers, a species in which the chromosomes are especially favorable for an investigation of the mitotic process — results which have obliged me to maintain a position different from that represented in the papers of Grégoire and Schreiner.

The most important of these papers, it seems to me, is that of A. and K. E. Schreiner on *Tomopteris* (1906*a*). In this species they have found an object, in which the whole maturation process of the chromosomes can be followed and demonstrated with an apparently indisputable clearness. After a comparison of their results on *Tomopteris* with the maturation process in other animals and plants (1906*a* and *b*), they find it very probable that (p. 474, 1906*b*) "dieser Process bei allen höheren organischen Wesen von einem gemeinsamen Gesetze geleitet wird, das ihm unter ähnlichen Verhältniszen ein ähnliches Gepräge aufdrückt, und zwar das Gepräge des '*Tomopteris*-Typus'"; and also that (p. 475) "die Zeit nicht fern ist, wo das 'Reduktionsproblem' von morphologischem Gesichtspunkte aus als gelöst angesehen werden darf."

I fully agree with A. and K. E. Schreiner, that the knowledge of the maturation process in *Tomopteris* is of great importance for our understanding of the same period in other organisms. Especially valuable seems to me their convincing demonstration of a parallel conjugation of the chromosomes in this species and their identification of the same process in so many other groups.[1] Of great value also are the demonstrations of Grégoire

---

[1] I have reason to believe that the conjugation process in *Enteroxenos* follows the type of *Tomopteris* more closely, than is shown in my figures (Bonnevie, 1906, fig. 33–42 and 159–162). During my first investigation of the spermatocytes of this species I both observed and figured stages, in which there was a parallel arrangement of thin chromatic threads, the ends of which were directed towards one pole of the nucleus; and it was, in fact, these pictures which made me join von Winiwarter

(1905) and of Schreiner (1906*a* and *b*) of the close resemblance between the chromosomes of very different species during the first maturation mitosis; and certainly, the presence of this same type throughout the whole animal (and plant) kingdoms cannot but give one the impression that (Schreiner, *loc. cit.*) "dieser Process bei allen höheren organischen Wesen von einem gemeinsamen Gesetze geleitet wird."

But what is the law that determines the behavior of the chromosomes during the maturation period?

In my opinion the answer to this question is not yet given, in spite of the apparently overwhelming evidence brought together in the works of Grégoire and Schreiner to demonstrate that the first maturation division is always a heterotypical one, and that this heterotypical character finds its explanation in the fact, that (Schreiner, 1906*a*, p. 44) "hier Ganzchromosomen, die nie mit einander eine Einheitlichkeit gebildet haben, von einander getrennt werden."

My results in *Nereis* will, however, clearly demonstrate that a "heterotypical" mitosis cannot be considered as identical with a reductional one; and I hope to show in the following pages that the problem of the reduction of the chromosomes is still entirely open to discussion.

My material consisted of a most valuable series of maturation and segmentation stages of *Nereis limbata*, collected by Professor E. B. Wilson at Woods Hole, in 1896–97. The sections as well as the uncut material, fixed partly in picro-acetic acid, partly in Flemming's fluid, proved to be still in perfectly good condition; and the material contains an uninterrupted series of stages, from the moment of fertilization to the four-cell stage, and also the later segmentation stages, up to fifteen and one half hours after fertilization, these, however, with intervals in their development of two to five hours.

(1901) in his hypothesis of a parallel conjugation. As I, however, found very few cells of the same appearance among the young oöcytes, I did not believe this to be the typical arrangement of the chromatin; and I therefore tried to find other stages showing the first traces of a parallelism between the thin threads. In the light of the chromosome relations in *Tomopheris* I now think it probable, that a reinvestigation of new material of *Enteroxenos* will give results somewhat different from those shown in my paper. (Added June, 1907.) This supposition is confirmed by Schreiner, 1907.

The maturation process in the egg of *Nereis* does not begin until fertilization has taken place, and the earliest stages contained in my material show the nuclear membrane still unbroken, while outside of it two small asters have made their first appearance. Within the large nucleus fourteen chromosomes are found scattered around, most of them, however, lying relatively near to the nuclear membrane.

The chromosomes appear in shapes, well known from other worms — *Allolobophora* (Foot and Strobell, 1905), *Tomopteris* (Schreiner, 1906*a*) and others — forming rings and crosses of different kinds; but they also very often appear in a more irregular shape. (See earl. proph. of 1st mat. div.; p. 62.)

A comparison of these different chromosome forms shows that they all are reducible to one and the same type — to a more or less elongated tetrad (Fig. 1, *a*) in which the four originally parallel elements may be arranged in different ways (Fig. 1, *b–g*).

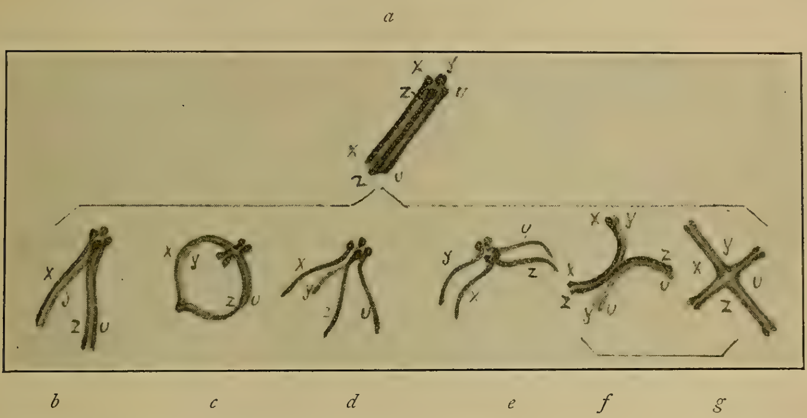
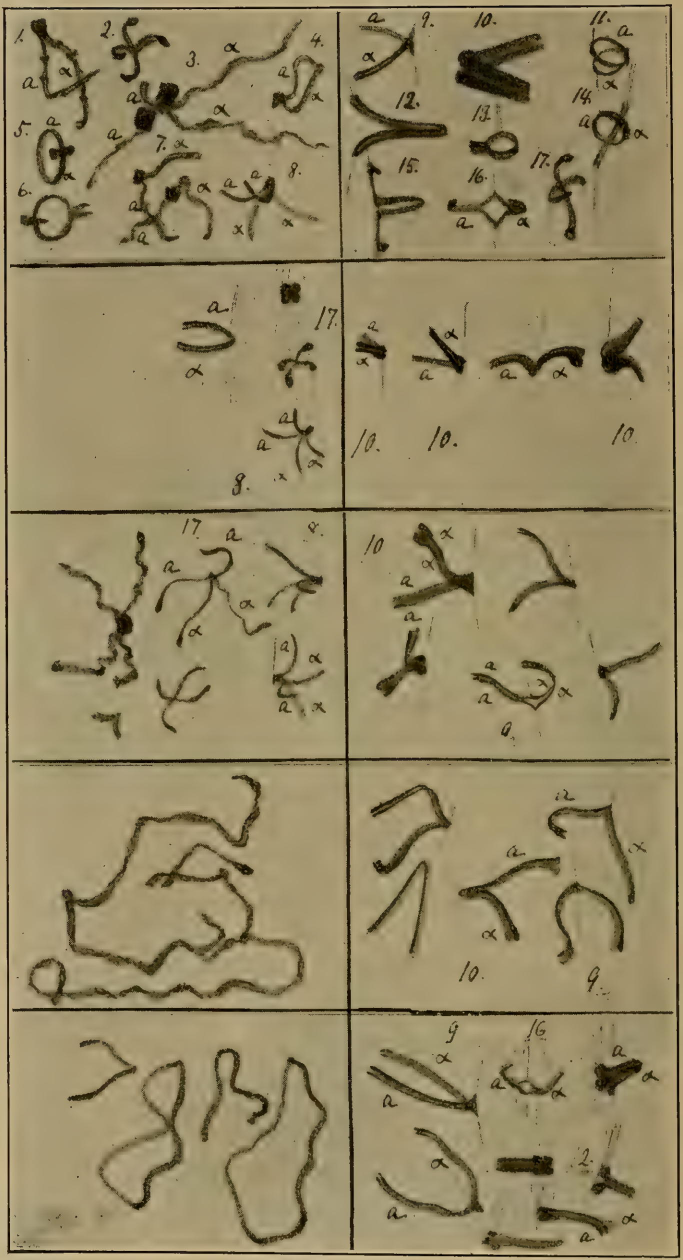


FIG. 1, *a–g*. Schematic illustration of the development of chromosomes from the original tetrad. Explanation in the text.

In most cases the four elements are combined in pairs, so as to give the appearance of two longitudinally split ribbons, connected at one (Fig. 1, *b*; chrom. 1, p. 62), or at both ends; in the latter case the chromosomes form more or less typical rings (Fig. 1, *c*; chrom. 4–6, p. 62).

In other tetrads we find the four elements connected at one end, but diverging from this point in different directions (Fig. 1, *d*). Such an arrangement gives rise to cross-shaped chromo-

Early Prophase | Later Prophase


1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
11.
12.
13.
14.
15.
16.
17.
a
α
17.
8.
10.
10.
10
17
9.
10
9.
10.
9.
9
16
2.

1st *Maturation* Division

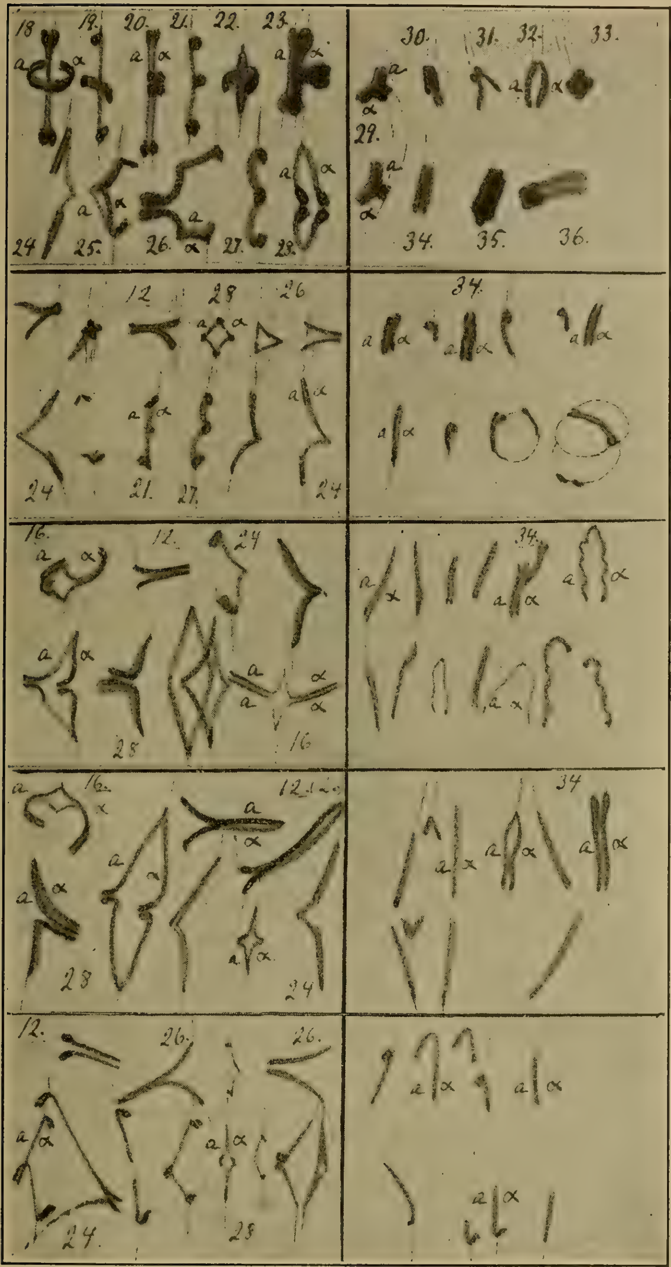
2nd *Maturation* Division

Early *Cleavage* Divisions (*1st–3rd*)

Cleavage Divisions *7½–11 h.* after Fertilization.

Cleavage Divisions *11–15½ h.* after Fertilization.

Early Anaphase Later Anaphase


18
19
20
21
22
23
a
α
24
25
26
27
28
29
30
31
32
33
34
35
36
12
28
26
24
21
27
24
34
16
12
24
28
16
16
12
34
28
24
12
26
26
24
28

1st
Maturation
Division

2nd
Maturation
Division

Early
Cleavage
Divisions
(*1st–3rd*)

Cleavage
Divisions
7½–*11h.*
after
Fertilization.

Cleavage
Divisions
11–15½ h.
after
Fertilization.

somes with four single arms, all of the same length and usually extending to one and the same side of their connecting point (chrom. 3, 8, p. 62).

Another kind of cross — double-armed ones — may also be derived from the original tetrad, its four elements being arranged in pairs, but combined in a different way at each end of the tetrad (Fig. 1, *f*. At the upper end the elements $x + y$ are diverging from $z + u$, at the other end $x + z$ remain parallel, diverging from $y + u$.) Through a flattening down of a figure, formed in this way we get a cross-shaped chromosome (Fig. 1, *g*), whose arms appear longitudinally split, and in which always two arms, lying opposite to each other, are of the same length. Sometimes this may be the case with all four arms, but more often we find a considerable difference between the two pairs. In many chromosomes this difference is so great that the short arms of the cross appear only like a pair of lateral projections on the middle of a longitudinally split ribbon; and from those forms there is a very short step to the chromosomes represented in Fig. 1, *b* and *c*.

Finally we may often find chromosomes consisting of two apparently separate halves (Fig. 1, *e*; chrom. 7, p. 62). Here also the origin of the chromosome may be traced back to a tetrad, and most easily through a transition form like that in Fig. 1, *b*, the arms of such a chromosome being divided along their longitudinal split.

A comparison of the whole chromosome group in a number of nuclei shows that these different forms are not characteristic of special chromosomes. I have found nuclei, in which all the chromosomes were cross-shaped, others in which two or three rings were present among the crosses, and again others, in which one or both of these forms were mingled with the more irregularly formed chromosomes.

Nor did I find any evidence in favor of the view, that the different forms of the chromosomes should represent different stages in their development. It seems more probable that the rings, the two kinds of crosses, the rodlike chromosomes, etc., arise simultaneously from the original tetrads and that their special shape is more a result of chance — possibly of their conditions within the nucleus — than of any individual character of the chromosome. The formation of rings seems, however, to be limited to chromosomes of a certain size.

At the time of the dissolution of the nuclear membrane the chromosomes are found to be slightly contracted, and while some of them regain their original tetrad-form, others remain like V- or horseshoe-shaped rods, longitudinally split and with a thickening at their middle point. Also the ring-shaped chromosomes usually retain their form, while the numerous crosses, found within the nucleus, are transformed into tetrads or V-shaped chromosomes. In only one case were two cross-shaped chromosomes found attached to an early spindle.

Considering the chromosomes of the prophase as different modifications of the tetrad, we will find that their attachment to the spindle-fibers is in all cases a terminal or a slightly subterminal one. (See lat. proph. of 1st mat. div., p. 62).

The unmodified tetrads are attached at one end, their longitudinally split halves being separated from each other (chrom. 12, 15).

The V- and horseshoe-shaped chromosomes are attached at their middle point, this representing one end of the original tetrad (chrom. 9, 10, 16).

The rings are placed horizontally[1] on the spindle and the fibers attached either at their transverse projections (chrom. 14) or at the point opposite to these (chrom. 11).

In each case the attachment is a terminal one, and the rings, as also the V-shaped chromosomes, are divided along a plane represented by their longitudinal split. In the above mentioned case in which I have seen cross-shaped chromosomes attached to the early spindle, the point of attachment seemed to be at their center, all four arms being bent in a direction away from the axis of the spindle (chrom. 17, p. 62). Considering the crosses as tetrads with four diverging elements, we find here also a terminal attachment of the fibers.

Besides the forms already mentioned I also found, in two or three cases, ring-shaped chromosomes placed in such a way on the spindle that they must be divided into two half-rings (chrom. 13, p. 62). In all of these cases, however, the rings were smaller than those found within the nucleus, and which we have seen placed horizontally on the spindle. I consider, therefore, that this is undoubtedly a secondary ring formation, caused by a subterminal attachment of the fibers to a tetrad-shaped chromosome.[1]

---

[1] In the following description the axis of the spindle is always supposed to have a vertical position.

The early prophase, in which the chromosomes are yet quite irregularly scattered on the surface of the spindle, is, according to the above stated facts, characterized by an arrangement of the chromosomes at right angles to the axis of the spindle.

This stage is followed by another, in which this horizontal position of the chromosomes is gradually changed into a vertical one, the daughter chromosomes being pulled towards each pole of the spindle (see earl. anaph. of 1st mat. div., p. 63).

During the time in which this separation takes place the chromosomes very often pass through a second cross-like stage (chrom. 18, 19, 22), the fibers being attached at or near the ends of the vertical arms of the cross. At first the horizontal arms are relatively long; a longitudinal split may be clearly visible in them, and they are often bent (in a direction away from the axis of the spindle) so as to form a more or less nearly closed ring (chrom. 18, 22).

---

[1] The behavior of the ring-shaped chromosomes in *Nereis* confirms my conclusion from *Enteroxenos* (Bonnevie, 1905, 1906) that the rings of the prophase cannot always be considered as identical with those of the metaphase. The prophase rings are in *Nereis* divided in their own plane, and during the separation of the daughter-chromosomes other rings are transiently formed from tetrads and V-shaped chromosomes; these metaphase rings are then sooner or later divided into two half rings.

According to a note in their latest work A. and K. E. Schreiner (1906*b*, p. 442) seem to have observed metaphase-rings in the first maturation division of *Enteroxenos*. This fact does, however, neither change nor contradict my results on the same species, that other rings are divided in their own plane and thus still appear as rings in the telophase.

In the same paper (Schreiner, 1906*b*, p. 444), is found the following phrase:

"Die Verfasser (Farmer u. Moore) meinen jetzt, wie Montgomery und Bonnevie, dass die bivalenten Chromosomen nicht durch Spaltung der in reduzierter Zahl vorhandenen Schlingen, sondern durch Zussammenbiegung derselben gebildet werden."

Because of this misleading account I want here once more to state my exact position with regard to these questions. I have described the bivalent chromosomes arising through a parallel conjugation of two homologous univalent ones — a view which is very different from that held by Montgomery and by Farmer and Moore. And with regard to ring-shaped chromosomes, I have shown that they may be formed in different ways, through an approach of the free ends of a chromosome (postsynapsis of *Enteroxenos*), or through a widening of a longitudinal split, while the two halves of the chromosome are still connected at their ends (cleavage division of *Enteroxenos* and many cases described in the literature). And further, that from the presence of ring-shaped chromosomes no conclusions can be drawn with regard to the nature of the mitosis, as it has been shown that rings may divide in two different planes.

The vertical arms of the cross on the other hand, are at first short, and their elongation evidently takes place at the cost of the horizontal arms. They are in most cases thinner than the horizontal arms (except their endpiece, if this is lying beyond the point of attachment of the fibers), and very often no longitudinal split can be seen in this part of the chromosomes (chrom. 19, 21, 24, 27). It is clear, however, from their earlier as well as from their later stages, that a doubleness is present even here; and when it is not visible, it may be due to the stretching of the vertical arms of the chromosomes.

Metaphase rings seem to be formed mostly from horseshoe-shaped chromosomes (chrom. 9, 16, 28). They are divided into two half rings. I have never observed a longitudinal split in these half rings; and an examination of a great number of chromosomes shows that the metaphase-rings of *Nereis* are to be directly compared with the cross-shaped chromosomes of the same stage, the space between the two branches of each half ring being identical with the longitudinal split in the vertical arms of the cross. Very often we find this space in the rings filled by an achromatic substance (like the "Zwischensubstanz" of the chromosomes of *Enteroxenos*);[1] and we find, indeed, all transition stages between the open rings and the thin vertically stretched arms of the crosses.

---

[1] (Added on the proof-sheet, June, 1907.) The existence of such a substance in the chromosomes of *Enteroxenos* is absolutely denied by A. and K. E. Schreiner (1907). They say (p. 12):

"Wir haben an unseren Präparaten vergebens nach dieser sehr eigenthümlichen Substanz gesucht, und es scheint uns unzweifelhaft, dass sich Bonnevie in diesem Punkt vollkommen getäuscht hat, indem sie bald die Spalte zwischen den Komponenten eines Doppelchromosoms, bald die Längslichtung in den Komponenten selbst, bald aber auch achromatische Verbindungen zwischen zwei oder mehreren Chromosomen als eine Kittmasse gedeutet hat."

In the face of this sweeping criticism I can only repeat that in *Enteroxenos*, as well as in *Nereis*, *Thalassema* and *Doris*, I have found the chromosomes of the maturation divisions, and especially those of the first, containing an achromatic substance which can be stretched out to a considerable width between the branches of the chromosomes.

In this respect the chromosomes of the maturation differ from those of other divisions in the same material.

If this substance has not been visible in Schreiner's preparations, it must depend upon its being dissolved or contracted.

After their separation the daughter chromosomes contract to relatively short and thick rods, in which the longitudinal split is in most cases clearly visible (see p. 63, lat. anaph. of 1st mat. div.). Sometimes the two halves of these chromosomes show a tendency to diverge from each other at their free end (chrom. 31); and once I have found a pair of daughter chromosomes (chrom. 29) in which a double longitudinal split seemed present — the one which is usually visible (separating *a* from *a*) and on the inside of the diverging halves of the chromosomes another split at right angles to the first one.

Such a tetrad-like appearance of the daughter chromosomes is found more often in the telophase (chrom. 35, 36). But I have not been able to decide with certainty, whether or not this appearance is due to a mere surface structure. An examination of the following stages, however, makes it very probable that these chromosomes ought to be considered as real tetrads.

Reviewing the different stages of the first maturation division, we find :

That the point of attachment of the daughter chromosomes corresponds to a point at (or near) the end of the original tetrad.

That the plane of division was represented by the longitudinal split of the rings and the V-shaped chromosomes of the early prophase — and

That the longitudinal split of the daughter chromosomes is identical with the space between the two arms of the V-shaped chromosomes.

* * *

What has been said about the chromosomes of the first maturation division may with some modifications be applied also to any of the following divisions up to fifteen and one half hours after fertilization.[1]

The elongated chromosomes are before each division placed horizontally on the (vertical) spindle, and — transiently appearing like rings, crosses, E-shaped chromosomes, etc., — they are before the separation of the daughter chromosomes carried into a position parallel to the spindle fibers.

---

[1] Whether the same type of mitosis may be found throughout the whole life of the animal, I am not yet able to say.

Up to eleven hours after the fertilization a longitudinal split may be clearly visible in the terminally (or subterminally) attached daughter chromosomes, this split being as in the first maturation division identical with the space between the two branches of a metaphase-halfring or, which is the same, between the two diverging arms of the horseshoe-shaped chromosomes of the prophase.

With regard to this period, therefore, it will be enough to mention the characteristics through which each division, or group of divisions, is distinguished from the other ones (comp. the chrom. of the different divisions on pp. 62–63).

SECOND MATURATION DIVISION.

In the eggs of *Nereis* there is no resting stage between the two maturation divisions, the formation of a new spindle taking place even before the first polocyte is fully separated from the egg-cell. The chromosomes, therefore, pass directly from the telophase of the first division into the prophase of the second, and no great changes are seen to take place in their structure (see p. 62, 2nd mat. div.).

The chromosomes of the prophase are mostly V- or horseshoe-shaped with a longitudinal split and attached by their middle point. There are however also found cross-shaped ones with four equally long arms being attached by their center on the surface of the spindle. In the metaphase we find the chromosomes arranged in a circle round the equator of the spindle, their form being through an approach of the diverging arms, transformed into a rodlike one. In a few cases I have seen a tetrad-like structure of these chromosomes (chrom. 12, p. 63) a fact which is in complete harmony with their genesis and with the appearance of a longitudinal split in the daughter chromosomes.[1]

---

[1] (Added on the proof-sheet, June, 1907.) With regard to a similar doubleness of the chromosomes shown by me (1905, '06) in *Enteroxenos*, A. and K. E. Schreiner express themselves as follows (1907, p. 18):

"Weder die Beobachtungen Bonnevie's von dem Vorhandensein einer solchen Doppelheit der Chromosomen, noch ihr Versuch, dieselbe mit der in der I. Reifungsteilung sichtbaren zu vergleichen, sind neu; vielmehr gibt es schon über diese Frage eine ganze kleine Literatur, die aber von Bonnevie nur geringe Beobachtungen gefunden hat."

They then mention the observations of Ed. van Beneden (1883) and Flemming

In the telophase the chromosomes grow longer and thinner, their longitudinal split often disappearing; and small drops of hyaloplasm are seen accumulating at the side of each of them, or between two neighboring chromosomes (lat. anaph. of 2d mat. div.). Through the growth and fusion of these vacuoles the female pronucleus[1] is formed; and the chromosomes, invariably adhering to the surface of the vacuoles, soon lose their staining power so that they cannot be distinguished within the resting pronucleus.

EARLY CLEAVAGE DIVISIONS.

In the early prophase of these divisions the chromatic substance of the nucleus appears in form of an irregular network, which, however, soon proves to consist of a number (28) of cross-shaped chromosomes, each with four equally long arms without any longitudinal split, and many of them with a conspicuous thickening at the center (earl. proph. of earl. cleav. div., p. 62).

These crosses, attached on the young spindle by their middle-point, are in later stages transformed into V-shaped, longitudinally split chromosomes — a transformation, which can only be due to an approach of two arms of the primary crosses on each side of their point of attachment.

The metaphase of these divisions differ from the maturation di-

(1887), both referred to in my final paper (1906, p. 390),—and besides these also those of Hof (1898) and Merriman (1904) on vegetative cell-divisions in plants.

According to observations, to be published in a following paper, I can now say, from my own experience, that this doubleness, occasionally mentioned as occurring outside of the maturation period is (with exception perhaps of Van Beneden's observations in the segmentation divisions of *Ascaris*), something quite different from the doubleness of the chromosomes at the end of the maturation period first found by me in *Enteroxenos* — and now also in *Nereis*, *Thalassema* and *Doris*.

What Hof (1898) has seen in "den eben fertig gebildeten Tochterkernen," is the same structure, which is later described by Merriman (1904). She, however, neither has, nor pretends to have, seen a real doubleness of the daughter chromosomes; and her comparison with the maturation phenomena consists in suggesting that also the doubleness so often described at this stage should be (p. 202) "due to the changing of the daughter-chromosomes from tubular structures into the quadripartite threads."

I must, therefore, insist upon the priority of having shown a doubleness of the chromosomes at the end of the maturation divisions. After the appearance of my preliminary account (1905), however, similar structures were shown to exist in *Myxine* (Schreiner, 1905), in *Ascaris mystax* (Marcus, 1905) and in *Dytiscus* (Schäfer, 1907).

[1] The male pronucleus also develops at the same time and in a similar way.

visions, as well as from the later cleavage divisions, practically all the chromosomes retaining their V- or horseshoe-shape and accordingly also their median point of attachment on the spindle (lat. proph. of earl. cleav. div., p. 62). In the early anaphase we therefore here find a greater number of ring-shaped chromosomes than in any of the other divisions.

The aspect of the later anaphase seems at first to form a striking contrast to that of earlier stages, the daughter chromosomes now being rodlike and terminally attached to the fibers. An explanation is, however, found in the fact, that all these chromosomes show a more or less clearly visible longitudinal split, which — as shown by the genesis of the chromosomes — is identical with the space between the two branches of a half ring.

In several cases this split extends all through the daughter chromosomes, so that they lose their V-shape, the two halves being quite separate from each other (chrom. 34, p. 63).

In the telophase the vacuoles are as a rule formed at the side of such a double chromosome or between two neighboring ones.

LATER CLEAVAGE DIVISIONS.

(*a*) *Seven and One Half to Eleven Hours After Fertilization.*

In the prophase of the later cleavage divisions we miss the cross-shaped stage of the chromosomes. They appear within the nucleus as longitudinally split ribbons (earl. proph., p. 62); and at the time of attachment to the spindle fibers they are, without exception, V- or horseshoe-shaped, being attached by their middle point.

The appearance of the later stages is, up to about nine hours after fertilization, very much like that of the early cleavage, the chromosomes retaining their prophase shape, until the daughter chromosomes are separated.

After this time, however, the aspect of the metaphase is changed through a tendency in the two arms of the V-shaped chromosomes to approach (chrom. 12 (26), earl. anaph., p. 63).

(*b*) *Eleven to Fifteen and One Half Hours After Fertilization.*

The change just mentioned, proceeds rapidly, and fifteen and one half hours after fertilization the metaphase of the mitosis has an appearance very much like that of the second maturation division. The 28 chromosomes, having in the prophase passed through a V-shaped stage (chrom. 9, 16, later proph., p. 62), are in the metaphase forming as many terminally attached tetrads, standing stiffly out from the spindle (chrom. 12).

Most of these tetrads show a considerable thickening of their four elements at their inner end; and a comparison with later stages of the mitosis makes it probable, that this phenomenon is in some causal connection with a dislocation of the point of attachment to the fibers. (See below, p. 74.)

In accordance with the rodlike shape of the chromosomes no open rings were found in the early anaphase. The doubleness of the daughter chromosomes is, however, still often indicated by narrow openings between their two branches during the separation of the daughter chromosomes (chrom. 28, earl. anaph., p. 63).

In the later anaphase no longitudinal split was visible in the daughter chromosomes.

These are the main facts observed in the material of *Nereis* now in my hand. The series of stages is, however, not yet complete, and I therefore prefer to postpone a general discussion of the bearing of my results, until I have had an opportunity of examining the nature of the mitosis also at the end of the germ track, and of comparing the chromosomes of *Nereis* with those of some other types.

On this occasion I only wish to draw the conclusions reached through the examination of the maturation and cleavage division in *Nereis*, and also to point out some questions, the answer to which will be of importance for an understanding of the chromosome relations in this species.

---

1. As already mentioned, the behavior of the chromosomes in all divisions in question is practically the same. Their point

of attachment, their plane of division, their typical changes of form during the separation of the daughter chromosomes and the longitudinal split of these chromosomes — these are characters common to all the divisions, taking place within fifteen hours after fertilization.

And yet, every division (or group of divisions) has its characteristic appearance, each representing one step in a series of transformations following the stage of the conjugation of the chromosomes.

The first maturation division is characterized by a great variability in the shape of the chromosomes and also in the time of the separation of the daughter chromosomes. No stage in this division can be considered as the metaphase of the whole mitotic figure, each chromosome passing through its characteristic stages independently of all the others, and without being placed in a typical equatorial plate. Characteristic also is a peculiarity in the (chemical or physical ?) structure of the chromosomes, making them appear less stiff and consistent than in other divisions.[1] Thus the daughter chromosomes are by their separation very often sharply bent, their ends forming right angles with each other (chrom. 18–25, 1st mat. div., p. 63), and there is also a marked tendency towards a spherical shape of the free ends of the chromosomes.

Taking the first maturation division as a starting point, all the changes in the mitotic figures going on within 15–16 hours after fertilization may be looked upon as a gradual return from these irregularities to the normal mitosis.[2]

The chromosomes regain slowly their original more rigid structure; and their cross-shape during the separation of the daughter chromosomes becomes less conspicuous to the same degree as the rigidity increases.

Of great interest are also the gradual changes of the chromosomes in the early prophase. The prophase of the first maturation mitosis is in most objects characterized by a more or less complete separation of the longitudinal halves of the chromosomes — and in *Nereis* this separation may take place along both longitudinal splits of the original tetrads. Also with regard to this character we find a gradual return to the general type. In the nuclei of the early blastomeres we still find a divergence of the four arms of the tetrad, although here without the variety in form characteristic of the first maturation division. Through an approach of each two arms of the prophase-crosses, the V-shaped chromosomes of the metaphase arise; and in later cleavage-divisions the V-shaped chromosomes of the early prophase are transformed into rod-like tetrads through a similar approach of their arms.

---

[1] Meves (1897) and Kingsbury (1902) have drawn quite similar conclusions from the form of the daughter chromosomes in the first maturation mitosis of amphibians.

[2] The second maturation division does not form a good link in this series of transformations, its whole appearance being more like the later cleavage-divisions than the earlier ones. This is, however, a natural consequence of the lack of a resting stage before this division, the changes of the chromosomes within the nucleus being of importance for their appearance during the mitosis.

2. Another result of general interest, reached through a comparison of the maturation and cleavage divisions in *Nereis*, concerns the point of attachment of the spindle fibers to the daughter chromosomes.

Although it seems, that the first connection between chromosomes and fibers always takes place at homologous points of the chromosomes, there is a very strong evidence in favor of the assumption that this point is changed during the mitosis.[1]

In the cleavage divisions all the chromosomes are derived from the cross- or V-shaped chromosomes of the prophase, being attached at their middle point. If, therefore, the point of attachment is a fixed one, a subterminal attachment of the daughter chromosomes would seem absolutely excluded. According to the degree of opening of the longitudinal split the attachment of the daughter chromosomes might be called a median or a terminal one, but any intermediate attachment would seem impossible. And yet, we almost always find some of these chromosomes subterminally attached — not so often in the early cleavage as in the maturation and in the later cleavage divisions, where more than half of the daughter chromosomes are often found to be subterminally attached (see p. 63).

An indication of the way in which this dislocation of the fibers takes place is given in the later cleavage divisions. The terminal attachment of the tetrads in the metaphase is identical with the median one of the V-shaped chromosomes in the prophase. The tetrads are, however, as it were, pressed against the fibers of the spindle, their proximal ends being thickened or curved (p. 62). The point of attachment of the fibers is in this way changed from a terminal into a subterminal one, the thickened ends of the tetrads being found again as the short branch of the subterminally attached daughter chromosomes. In some cases it seems as if the point of attachment during the early anaphase is still further removed from the end of the chromosome — a dislocation probably due to the fact, that the median part of the daughter chromosomes makes less resistance against a separation than the ends. (See the triangular chrom.; sec. mat. div., p. 63).

---

[1] This probability was, from another point of view first suggested by Schreiner, 1906*b*, p. 433.

3. What is the bearing of the chromosome-relations in *Nereis* with regard to the assumption of a universally existing reduction division?

In the papers of Schreiner (1904, 1906*a* and *b*), Grégoire (1905) and Montgomery (1905) the universality of a prereductional mode of maturation is based upon the similarity between the chromosomes of different species, and more especially on the general appearance of a "heterotypical" mitosis in the first maturation division.

Grégoire (1905) in his valuable review of the literature concerning the maturation divisions in plants and animals, tries to explain the phenomena in question as following his "hétérohoméotypique" scheme — the first maturation division being a heterotypical, the second a homeotypical one.

His provisional definition of these two modes of mitosis and his opinion with regard to their bearing is found in the following sentences (*loc. cit.*, p. 254).

"Pour la période qui nous occupe, la caractéristique de l'hétérotypie consiste dans la division longitudinale anaphasique ; la caracteristique de l'homéotypie réside en ce que les chromosomes-filles de cette cinèse sont preparés dès la cinèse précédente par une division longitudinale."

". . . ce schéma, . . ., s'oppose directement au processus *postréductionnel*, mais il laisse ouverte la question du processus *préréductionnel* et du processus *eumitotique*."

(P. 362): "Disons-le dès maintenant, c'est le *schéma préréductionnel* dont nous espérons démontrer la réalité."

A. and K. E. Schreiner who in their main results fully agree with Grégoire, characterize the heterotypical appearance of the first maturation division in *Tomopteris* as follows (1906*a*, p. 44): "Geht man . . . auf eine genauere Betrachtung des Verhaltens der Chromosomen in den Reifungsteilungen und auf einen Vergleich desselben mit dem Verhalten der Chromosomen in anderen Teilungen ein, so kann es ja keinem Beobachter entgehen, dass die I Reifungsteilung bei fast allen Objekten, wo es gelungen ist, die Struktur der Chromosomen zu analyzieren, einen gemeinsamen Typus zeigt, der sich von dem Typus aller anderen Teilungen in charakteristischer Weise unterscheidet; und zwar besteht der Unterschied darin, dass sich die Schwesterelemente der einzelnen Chromatinportionen bei dieser Teilung schon lange vor dem Eintreten der Mitose . . . in weiter Ausdehnung von einander trennen und während der ganzen Pro- und Metaphase eine viel gröszere Selbständigkeit zeigen, als in irgend einer anderen Teilung der Fall ist. Auch sind die Verbindungen zwischen den Schwesterelementen in dieser Teilung von ganz anderer Art als bei allen anderen Teilungen." . . . "Es scheint uns, dass das Auftreten dieser eigenthümlichen Bilder der Chromosomen während der ersten Teilung nach dem Eintreten der Zahlenreduktion nur dadurch befriedigend erklärt werden kann, dass hier Ganzchromosomen, die nie miteinander eine Einheitlichkeit gebildet haben, von einander getrennt werden."

These considerations represent, according to the authors, the main reasons for their assumption of a prereductional maturation in *Tomopteris*, as it "allein aus der Betrachtung der verschiedenen Längsteilungen kaum möglich (ist) zu einer endgültigen Lösung dieser Frage zu gelangen."

Their assumption of a universality of this mode of maturation, they base upon the general appearance of the "*Tomopteris*-typus" also in other species. So much weight is laid upon this similarity, that the heterotypical character of a few chromosomes is considered a sufficient proof of a reductional nature of the mitosis. Thus in their description of the maturation divisions of *Myxine*, we find (1906*b*, p. 459):

"Nach der Einstellung in die Teilungsebene zeigen die Chromosomen in gewissen Fällen die für die I Reifungsmitose so charakteristischen, in die Äquatorialebene fallenden Verdikkungen und seitlichen Ausläufer, die sicher beweisen, dass es die Spalthälften der bivalenten Schlingen, die Konjuganten, sind, die hier getrennt werden."

The above cited sentences of Grégoire and Schreiner contain the view which forms the basis in their generalizations with regard to the reduction division.

Judging the chromosome relations in *Nereis* from the same point of view, we should find a reduction of the chromosomes taking place not only in each maturation division but also in each of a whole series of cleavage divisions. The early separation of the daughter chromosomes, the heterotypical shape of the chromosomes in metaphase, and their doubleness in the anaphase, are characters common to all these divisions.

This result is so clear and its consequences are so evident, that a further discussion upon this point would seem unnecessary. Before the question about the nature of the maturation divisions can be considered ripe for new generalizations, it will be necessary to widen the base of our investigations to a comparative study not only of the maturation process itself, but also of the changes of the chromosomes during the time following this period.

Though, however, the main base of the modern generalizations is removed through the knowledge of the chromosome relations in *Nereis*, it is not therefore excluded, that a reduction division may exist in this species as well as in others; and we now finally turn to the question:

4. How is the maturation process in *Nereis* to be understood?

In answering this question three different possibilities must be considered, all of which have already been applied for the maturation process in other species.

(*a*) One of the maturation divisions is a reductional one, separating chromosomes, which have conjugated at an earlier period.

(*b*) Both maturation divisions are to be considered as ordinary mitoses, the conjugating chromosomes having fused completely with each other (Boveri, 1890).

(*c*) The conjugating chromosomes do not separate again; but their fusion may proceed so slowly that the appearance of the following divisions is influenced by the doubleness of the chromosomes (Bonnevie, 1905, '06).

Of these three possibilities only the first one will be treated fully in this paper.

According to the concordant results of modern investigators the two longitudinally split halves of the chromosomes in the prophase of the first maturation mitosis (*a* — *a*, p. 62) are to be considered as two conjugating chromosomes united to form a bivalent one.[1]

If, therefore, these two halves were separated from each other in the first maturation division, then this division must with great probability be considered as a reductional one, and all the similar structures in the following divisions would have to be explained in some other way.

Such a conclusion might, in *Nereis*, as in so many other objects, easily be drawn, if the chromosomes of the early prophase are compared with those of the early anaphase; it would, indeed, seem very natural to consider the thickening in the equator of metaphase (or anaphase) chromosomes as identical with that connecting their two halves in the early prophase. But between these two stages there is another, the stage in which the chromosomes are first attached on the young spindle,[2] showing that the long axes of the chromosomes are placed at right angles to the axis of the spindle, that they are attached to the fibers at the connection point between their two halves and divided in a plane represented by their longitudinal split, and finally, that their position parallel to the spindle fibers is secondary — reached during the separation of their daughter chromosomes.

If, therefore, the assumption is correct, that each half of the chromosomes of the prophase represents one of the conjugating chromosomes, then the same must be true of the daughter chromosomes; and *the first maturation division is certainly not a reduction division.*

---

[1] It makes here no difference, whether the conjugation of the chromosomes is considered as a parallel one or as having taken place "end to end"; in each case the connection between the two conjugates is supposed to be of the same kind at a stage directly preceding the maturation divisions.

[2] As will be shown in my final paper, there is no escape from this fact through the suggestion that I should have "confondu les stades" (Gregoire, 1904, p. 307).

The possibility, however, of the second maturation division of *Nereis* being a reductional mitosis, is not absolutely excluded, although the only reason for accepting this view must, as far as I can see, be sought in a preconceived assumption in favor of such a mode of maturation.

The chromosomes of the prophase show in the second maturation division, as in the first, and in the early cleavage divisions, two longitudinal splits, one of which is identical with the split of the daughter chromosomes of the first division, the other being a new formation generally not appearing till in the prophase of the second but sometimes indicated as early as in the anaphase of the first division. In most cases it seems impossible to decide with absolute certainty which of these splits represents the division plane of the chromosomes.

Supposed, however, that a separation of the conjugated chromosomes should take place in this division, then the thorough resemblance in the genesis and the whole appearance of the chromosomes during the maturation and cleavage divisions must be considered as a mere chance. The longitudinal split of the daughter chromosomes, would have a different meaning in each of the maturation divisions and in the cleavage; in the first maturation division it would mean the split between the conjugated chromosomes, in the second it must be explained in some other way — most likely, perhaps, as a precocious splitting for the next division; in the cleavage divisions, finally, the longitudinal split is certainly not to be seen in connection with the following division as it is shown to be identical with the space between the two branches of the V-shaped chromosomes of the prophase. In the same way all the other stages in the development of the chromosomes would, in spite of their detailed resemblance, have to be explained in different ways.

Considering, on the other hand, the fact that in this species, so favorable for an examination of the chromosomes, no evidence at all is found, which might establish a proof of the existence of a reduction division — it seems to me more natural to look upon the conformity in the behavior of the chromosomes as an expression also of a corresponding series of changes going on within them during each mitosis.

Whatever, therefore, the meaning may be of the different structures of the chromosomes, they ought, I think, to be looked upon from one and the same point of view in each of the maturation divisions as in the cleavage, and the results reached in any of these divisions may be used for an explanation of similar structures in the others.

According to this view, I consider the question of a reduction division in *Nereis* as lying outside of the actual discussion until a sufficient proof for its universality is established in other species.

The questions to be settled with regard to *Nereis* concern the two other possibilities, mentioned above — whether or not the conjugating chromosomes have fused completely before the maturation divisions.

As I said before, I have not yet the material for a definite answer of this question ; and it will be the aim of my following investigations to procure such material through a comparative study of the chromosomes of the germ track in *Nereis* and those of other species.

With our present knowledge of the chromosome-relations in *Nereis* the evidences in favor of each of these views seem to balance each other.

If we take our starting point in the prophase of the first maturation division, considering the two longitudinally split halves (*a* and *α*, pp. 62–63) of the chromosomes as the conjugates being terminally attached to the spindle, then it would seem natural to look upon the quite similarly shaped chromosomes of the following divisions from the same point of view. The gradual changes in the whole appearance of the mitosis within the first 15 to 16 hours after fertilization must then be considered as the expression of a slowly proceeding fusion of the conjugating chromosomes (*a* and *α*), their tendency of a divergence during the prophases gradually decreasing, and their fusion during the anaphases becoming always more complete.

If on the other hand, we begin with the early cleavage divisions, where all the chromosomes are V- or horseshoe-shaped and with a median attachment to the fibers — then it would seem more natural to consider the longitudinal split of the anaphases merely as an unusually narrow space between the two arms of V-shaped daughter chromosomes, and the cross-shaped chromosomes of the prophases as arisen through a precocious separation of the daughter chromosomes, connected or crossing each other on their middle point. The same view must be held also with regard to the maturation and the later cleavage divisions, the tetrads, so often met with, being considered as merely morphological structures, arisen through an approach of the two arms of a V, that is, their two longitudinally split halves, *a* and *a* must be looked upon as forming one continuous ribbon, sharply bent on its middle point. The point of attachment of the chromosomes would, from this point of view, in all divisions be a median one, the many cases in which a terminal attachment seems to be shown, being explained as artefacts.

As will be seen from the above, each of these interpretations meets with difficulties, which at the present state of our knowledge interferes with the acceptation of any of them.

In order to solve these difficulties it will be of great importance to follow the changes in the mode of attachment of the chromosomes throughout the whole germ track — and especially to compare the chromosome-relations of species in which a median attachment of the chromosomes seems to be predominant (*Tomopteris*, *Salamandra*, etc.), with those of other species, in which the chromosomes are terminally attached to the fibers.

In my following papers I shall publish my first results of such a comparative study, and even if the solution of the problem of maturation is still far away, I hope to be able to throw some new light on the question.

Columbia University,
March, 1907.

LITERATURE.

Beneden, Ed. van.

'83 Recherches sur la maturation de l'œut et la fécondation. Arch. de Biol., T. 4.

Bonnevie, Kr.

'05 Das Verhalten des Chromatins in den Keimzellen von Enteroxenos östergreni. Anat. Anz., Bd. 26.

'06 Untersuchungen über Keimzellen. I. Beobachtungen an den Keimzellen von Enteroxenos östergreni. Jen. Zeitschr., Bd. 41.

Boveri, Th.

'90 Zellenstudien. III. Ueber das Verhalten der chromatischen Kernsubstanz bei der Bildung der Richtungskörper und bei der Befruchtung. Jena.

Flemming, W.

'87 Neue Beiträge zur Kenntniss der Telle. Arch. f. mikr. Anat., Bd. 29.

Foot, K., and Strobell, E. C.

'05 Prophases and Metaphase of the First Maturation Spindle of Allolobophora fœtida. Amer. Jour. of Anat., Bd. 30.

Grégoire, V.

'04 La réduction numérique des chromosomes et les cinèses de maturation. La Cellule, T. 21.

'05 Les résultats acquis sur les cinèses de maturation dans les deux règnes. La Cellule, T. 22.

Hof, A. C.

'98 Histologische Studien an Vegetationspunkten. Bot. Centrbl., Vol. 76.

Marcus, H.

'05 Über Samen- und Eibildung bei Ascaris mystax. Sitz. Ber. Ges. Morph. Phys. München.

'06 Ei und Samenreife bei Ascaris canis (Werner) (Asc. mystax). Arch. mikr. Anat., Bd. 68.

Merriman, M. L.

'04 Vegetative Cell Division in Allium. Bot. Gaz., Vol. 37.

Meves, F.

'97 Ueber die Entwicklung der männlichen Geschlechtzellen von Salamandra maculosa. Arch. f. mikr. Anat., Bd. 48.

Montgomery, Th.

'05 The Spermatogenesis of Syrbula andLycosa, with general Considerations upon Chromosome Reduction and Heterochromosomes. Proc. Acad. Nat. Sc. Phil.

Schäfer, Fr.

'07 Spermatogenese von Dytiscus. Zool. Jahrb. Abth. f. Anat. u. Out., Bd. 23.

Schreiner, A., und K. E.

'04 Die Reifungsteilungen bei den Wirbeltieren. Anat. Anz., Bd. 24.

'05 Ueber die Entwicklung der männlichen Geschlechtszellen von Myxine glutinosa. Arch. de Biol., T. 21.

'06*a* Neue Studien über die Chromatinreifung der Geschlechtszellen. Arch. de Biol., T. 22.

'06*b* Neue Studien über der Chromatinreifung der Geschlechtszellen. II. Die Reifung der männlichen Geschlechtszellen von Salamandra maculosa (Laur.), Spinax niger (Bonap.) und Myxine glutinosa (L.).

'07 Neue Studien über die Chromatinreifung der Geschlechtszellen. IV. Die Reifung der Geschlechtszellen von Exteroxenos östergreni Bonn. Vidsk. Selsk. Skr. Kristiania.

Wilson, E. B.

'92 The Cell-Lineage of Nereis. Journ. of Morphol., Vol. 6.

v. Winiwarter, H.

'01 Recherches sur l'ovogenèse el l'organogenèse de l'ovaire des Mammifères. Arch. de Biol., T. 17.

To the Plates, pp. 62–63.

The chromosomes represented in these plates are selected from among a great number of camera drawings which will be published in a following paper.

They are all drawn to the same scale, enlargement about 3,500 : 1.

The chromosomes of the first maturation division are continuously numbered ; some of these numbers being applied also to chromosomes of the later divisions, showing essentially the same structure as those of the first.

The letters *a* and *a* are applied to the morphologically distinguishable halves of the chromosomes, demonstrating their reappearance in each group of divisions, but without any interpretation concerning the meaning of these structures.

The relatively larger size of the chromosomes of the later cleavage divisions (7 to 15 h. aft. fertil.) may be due to their fixation in Flemming's fluid, while those of the maturation and the early cleavage are fixed in picro-acetic acid.

THE SACRUM OF THE LACERTILIA.

ROY L. MOODIE,

THE UNIVERSITY OF CHICAGO.

The question of the morphology of the transverse processes of the sacral vertebræ of the *Lacertilia* seems never to have been definitely settled. There are extremely diverse statements in our various works on zoölogy concerning the exact nature of these processes. So far as I have been able to determine no one has ever taken up the study of the young stages of the lizards in order to determine this point. At the suggestion of Dr. S. W. Williston I have undertaken such a study while engaged in clearing the young and adult stages of a number of reptiles in the course of an extensive investigation on the epiphyses of the Reptilia.

The problem as it presents itself is whether or not the *Lacertilia* possess sacral ribs. If they do, there should be separate centers of ossification for these elements and we may confidently expect to find them in the embryonic condition. If there are no ribs, there should be no separate centers of ossification nor would sutures of separation of the ribs from the centra persist in the young of the lizards. The question as to whether the lizards ever had sacral ribs is not fully discussed. If the forerunners of the lizards had such ribs there would probably be a cartilaginous remnant of them in the embryo.

The material investigated includes the young and adult stages of representatives of five families of the *Lacertilia*, viz.: (1) Chameleonidæ — *Chameleon owenii* Grey, two specimens, one young and one adult from Batanga, German East Africa. (2) Iguanidæ — *Iguana* sp. ?, one young specimen from Mexico. *Phrynosoma douglassi hernandesi* Girard, three specimens, two young and one adult, taken in Natrona Co., Wyoming, this past summer by the writer. *Sceloporus* sp. ?, one young specimen from Mexico. *Sceloporus chrysosticus* Cope, nine specimens, eight young and one adult from Zopopan near Jalisco, Mexico on the semi-arid upland plains collected by W. L. Tower. (3) Teiidæ — *Cnemidophorus sexlineatus* Linné, ten specimens, one adult and nine embryos from Mexico. (4) Agamidæ — *Draco volans* Linné, one adult specimen from the East Indies. (5) Helodermatidæ — *Heloderma suspectum* Cope, one adult specimen.

The specimens were cleared by the Schultze (1) method recently recommended by Dr. Mall (2) and more fully set forth by Hill (3). The method was adopted at the suggestion of Dr. Lillie and experiments have been made on clearing the young and adult stages of all groups of the Vertebrata except the fishes. The methods used are essentially those followed by Dr. Mall and need not be enumerated here. For a complete statement of the method the reader is referred especially to Hill's paper where Dr. Mall's methods are fully outlined. The Schultze method is an excellent one for demonstrating the intimate relations of the bones and cartilages of small animals and deserves to come into more general use as a method for laboratory demonstration.

Among the many recent writers on the lizards Friedrich Siebenrock seems to be the only one who has a definite conception of the true nature of the transverse processes of the sacral vertebræ of the *Lacertilia*. Cope with all of his keen insight and his wide acquaintance with fossil and recent reptiles seems not to have comprehended the unique character of the lacertilian sacrum. This seems the more remarkable since Cope did more on the lizards than any other naturalist. Cope's observations, however, are in some respects hasty and much of his work will need thorough revision. As an example of this he states on page 163 of his large work on the reptiles of North America, in speaking of the crocodiles : "There are two sacral vertebræ and no sacral ribs." But in the sacrum of the crocodiles there are no transverse processes and there *are sacral ribs.*

Siebenrock in writing on the skeleton of *Lacerta simonyi* Steind. (4) makes the following statement concerning the transverse processes of the lacertilian sacral vertebræ : "Die Frage über die morphologische Bedeutung der Querfortsätze an den Sacralwirbeln der Saurier sieht noch einer entsheidenden Lösung entgegen. Nach Gegenbaur (5) könnte man sie sowohl mit den praesacralen Rippen als auch mit den postsacralen Querfortsätze vergleichen, so dass die Homologie zwischen Rippen und Querfortsätze ergeben wurde. Hoffman (6) glaubt jedoch annehmen zu dürfen, dass dieselben selbständig ossificiren und daher den Rippen entsprechen, obwohl der von Hoffman untersuchte *Monitor*-Embryo in der Entwickelung schon zuweit vorgeschritten war, um die Trennung der Querfortsätze vom Wirbeln constatiren zu können. Diese Trennung kann sich aber sogar an ausgewachsenen Thieren erhalten, wie von mir in drei Fallen und zwar an einem Skelete von *Hoplurus*, *Tropidurus*, und *Uromastix* wahrgenommen wurde. Denn der erste Sacralwirbel besitzt Rippen anstatt der Querfortsätze, welche dem Wirbel nicht allein durch eine Naht wie bei den Krokodilen und Schildkröten getrennt werden, sondern mit demselben sogar gelenkig verbunden sind."

In his contribution to the subject of vertebral assimilation Siebenrock (7) describes several cases such as he mentions in the above quotation and figures the condition in the sacra of *Uromastix spinipes* Merr. and *Lacerta simonyi* Steind. where the first sacral vertebra as Siebenrock calls it but which is in reality a posterior dorsal, bears a rib. Cope (8) likewise, mentions such a case as occurring in the vertebral column of *Phrynosoma* and Siebenrock has found the same condition in that genus. Such a condition, however, cannot be interpreted to mean three sacral vertebræ as Siebenrock believes. The vertebra assimilated is not a morphological sacral but merely a functional one and on that account cannot be called a true sacral. Such a condition as Siebenrock describes is of frequent occurrence among the other reptiles. The appearance of a rib in this situation is not a very remarkable occurrence since there are ribs in this situation in the primitive vertebrates and the occurrence of this rib in the lizards may confidently be regarded as a persistence of the embryonic condition in which the sacral ribs remain as vestiges. In the sacrum of *Lyriocephalus* Siebenrock (12) describes a vestige which may be interpreted to be a remnant of the sacral rib.

A close study of my material clearly shows that there are no sacral ribs in the modern lizards. The ilia are always attached directly to the *transverse processes* of the two sacral vertebræ. In the young specimen of *Chameleon owenii* Grey the transverse processes of the two sacral vertebræ are of equal size and are very short. They arise broadly from the centra of the vertebræ. The same may be said of the adult specimen. In the *Phrynosoma* specimens a nearer approach to the ancestral condition of the vertebrate sacrum is seen. Gegenbaur (13) is of the opinion that the sacrum of the vertebrates was primitively of but one vertebra

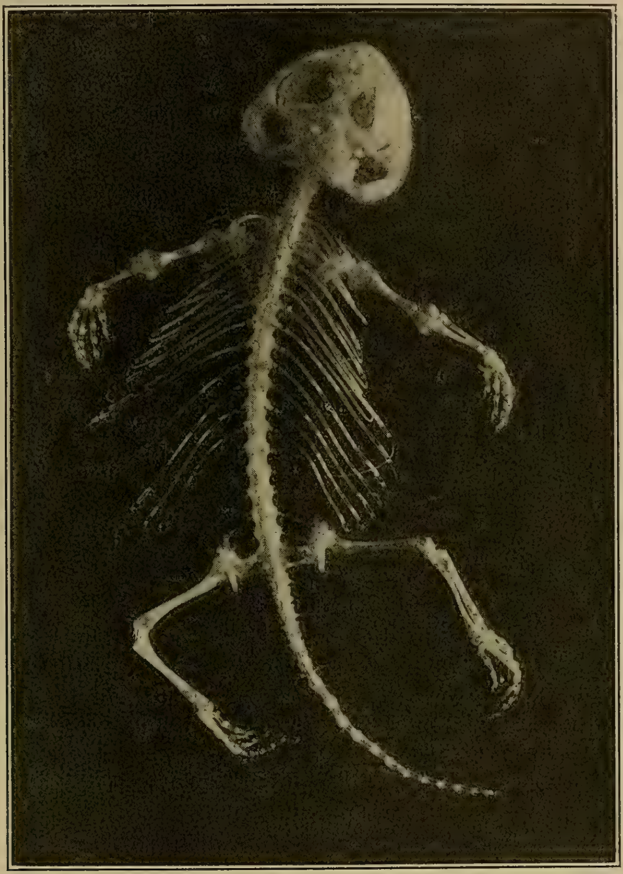


FIG. 1. Skeleton of *Phrynosoma douglassi hernandesi.* Girard.

as is found in the modern amphibians. Such a condition is what would be expected. In *Phrynosoma* the first sacral vertebra bears large stout transverse processes with expanded ends. The second sacral bears. much smaller processes but they are still larger than the processes on the succeeding pygals. These likewise arise from broad bases. The disparity in size between the two pairs of processes of the sacrals is such that the anterior pair appears to bear all of the burden of support (Fig. 1). In the specimen of *Sceloporus* the transverse processes are of more nearly equal size but are longer than in the *Chameleon*. In *Draco* the processes are stout and end broadly. In *Iguana* the transverse processes of the sacrals are of equal strength and have broad ends. In *Heloderma* there are broad transverse processes supporting the pelvis. In *Cnemidophorus* the sacral vertebræ bear stout transverse processes of which the anterior pair is the larger.

One would expect to find a suture of separation between the processes and the sacral centra of young lizards did such a separation really exist but even in the youngest of my specimens, *Sceloporus*, which are two days old and in which the epiphyses are just beginning to appear as minute centers of ossification, there is not the slightest indication of any separation between the sacral centra and their transverse processes. But we have more positive evidence still, that there are no sacral ribs in the lizards. In an embryo of *Cnemidophorus sexlineatus* Linné which measures 24 mm. from the tip of the snout to the base of the tail and in which the diaphyses of the limb bones are only one half ossified and the epiphyses have not appeared at all, the broad connection of the transverse processes to the centra of the vertebræ is clearly apparent. Furthermore the ossification of the processes is seen to be proceeding outward from the body of the vertebra into the transverse process so that there is no chance for a separate center of ossification in the processes. It can thus be very definitely stated that the Lacertilia occupy an isolated place among all other known reptiles in *not having any sacral ribs whatever*.

In nearly all of our modern text-books on zoölogy the statement is made that there are sacral ribs in the lizards and in none is a statement made to the contrary. Even Huxley (9) in his work on the anatomy of vertebrated animals states that there are sacral ribs but does not discuss the matter. Parker and Haswell (10) in their "Text-Book of Zoölogy" make the statement: "The sacral vertebræ have short and strong expanded processes — *the transverse processes* — which abut against the ilia, these are separately ossified and are to be looked upon as sacral ribs." Gadow (11) in his work on "Amphibia and Reptiles" says: "The pelvis is attached to two vertebræ by means of several ribs." I have given in the bibliography, under (10), a complete list of our general works on zoölogy in which there is any statment made concerning the sacral ribs of the Lacertilia. They all agree pretty well that there are sacral ribs.

In order to be sure that there are sacral ribs among our living reptiles other than the lizards I have investigated both the young and adult stages of the turtles, crocodiles and *Sphenodon*. In a young turtle, *Chelydra*, 44 mm. in length, the separation between the vertebral centra and their sacral ribs is clearly apparent. In a young alligator, six inches in length, the sutures between the sacral ribs and the centra are clearly seen as they are also in a specimen something over four feet in length. The sutures persist in the adult of the alligator and are found in the young and adult of the *Gavialis gangeticus* Gmel., thus completely disproving Cope's statement that there are no sacral ribs in the crocodiles. In *Sphenodon* the sacral ribs are distinct. We know that among the Dinosauria the sacral ribs did not fuse with the centra until late in life. There is in the Field Museum a specimen of a young *Morosaurus*, as identified by Mr. Riggs, which is of considerable size, and yet the sacral ribs are clearly separated from the centra. Marsh has figured a similar condition in the sacrum of *Morosaurus lentus* on Plate XXXIII. of his "Dinosaurs of North America." Hatcher in his paper on *Haplocanthosaurus* (14) gives a lengthy and very interesting discussion of the sacral ribs in the Dinosauria. He expresses it as his opinion "*that there are no true sacral ribs homologous with these elements in the tailed amphibia and that the so-called ribs are really homologous with the parapophyses or inferior branches of the transverse processes*."

But Mr. Hatcher is mistaken in his conception of the homology of these elements above mentioned. If it is true, as he states it is, that the sacral ribs (parapophyses, Hatcher) and the transverse processes of the caudal vertebræ arise from distinct ossificatory centers in the sauropod dinosaurs then we have in these animals a primitive condition and especially as regards the caudal ribs, recalling, as it does, the condition which exists in the modern tailed amphibians (*Menopoma, Necturus*). McGregor (15) has described separate caudal ribs in the Phytosauria and also describes free sacral ribs for these animals. In the young specimen of *Chelydra* referred to above the caudal ribs are clearly distinct and are separated from the centra by sutures. In the Ichthyosauria (16) the ribs were free throughout the entire length of the vertebral column. Dr. Williston tells me that he has found free caudal ribs in certain plesiosaurs. In the plesiosaurs, also, Dr. Williston has recently discovered free sacral ribs. From the above enumeration it is clear that caudal ribs are not rare among the reptiles and there can be no doubt, it seems to me, that when a free structure occurs in the sacrum it can be readily homologized with both the presacral and postsacral ribs. In the primitive condition the ribs were not differentiated into dorsals, sacrals and caudals and they varied but little in size. The dorsal and sacral ribs are retained in the majority of reptiles having become functional through use or other cause while the caudal ribs which had no real function to perform have become atrophied or vestigial. Sacral ribs without doubt exist in the dinosaurs. One striking peculiarity of the sauropod dinosaur sacrum is the elongate character of the diapophyses which in many cases serve to aid the sacral ribs in the support of the ilium. This condition obtains in the sacra of *Apatosaurus* and *Brachiosaurus* at least. The presence of these diapophyses led Hatcher, without doubt, to contend that the sacral ribs were parapophyses. But so far as I can see the presence or absence of diapophyses could have no effect whatever on the character of the sacral ribs.

Paleontology helps us not at all in determining the primitive condition of the modern terrestrial lizard sacrum. Of *Paliguana* (17) from the Triassic of South Africa only a fragmentary skull is known and from this form in the Trias to *Iguanavus* (18) in the Laramie Cretaceous, a period representing a lapse of millions of years, our knowledge of the terrestrial lizards is a complete blank. Tertiary lizards are represented for the most part by very fragmentary remains and belong, according to our best authorities, to existing families or to families only recently extinct so that they offer no differences in the morphology of the sacrum from the existing forms.

Nor do the allies of the lizards, the mosasaurs, aigialosaurs and dolichosaurs, offer any clue to the primitive condition of the sacrum. In the Mosasauria, according to Dr. Williston, there is never any sacrum, but the ilia are attached directly to the transverse processes of the vertebra which is either the second or third pygal, at least in one genus (19).

The Dolichosauria differ from the Mosasauria in that the former possess a sacrum of two vertebræ (20) but so far as I have been able to determine there has never been made out in these animals any sacral ribs. In *Adriosaurus* (21) no sacral ribs can be detected because the animal is preserved on its back and no attempt has been made, so far as I am aware, to determine the presence or absence of sacral ribs in this specimen. In *Acteosaurus* (22), however, the sacrum is well exposed but shows no evidences of sacral ribs. In *Dolichosaurus* (23) Owen says: "The extremities of the sacral pleuropophyses come into contact in the *Dolichosaurus* but do not coalesce." From Owen's figure it is difficult to make out just what the condition is in the sacrum of this form. The artist has certainly drawn sacral ribs in the figure but this may have been due to fracture or to a misconception on the part of the artist. Owen makes no statement of any sacral ribs.

In the Aigialosauria from the Cretaceous of Lesina the same conditions hold in the sacrum as we have described for the other forms. Gorjanovic-Kramberger (24) figures a skeleton of *Aigialosaurus* which gives no evidence of any sacral ribs nor does the author mention any ribs as occurring in the specimen. The evidence from *Opetiosaurus* (25) is purely negative since the sacral region of the specimen was in a very poorly preserved condition.

It is an interesting question for speculation just why and how such a condition as we have described should obtain in the lizards. It seems most probable that the Lacertilia constitute a branch which came off in pre-Triassic times from some primitive diapsid stem in which the sacral ribs were functional and that later the ribs from some unknown cause became atrophied.

In conclusion I wish to express my sincere thanks to Dr. Frank R. Lillie, under whose direction this work was done, for his kindly interest in my studies and for his advice. To Mr. W.

L. Tower and Mr. R. E. Scammon I am under obligations for the material which they have very kindly placed at my disposal.

BIBLIOGRAPHY.

1. **Schultze, O.**
'97 Ueber Herstellung und Conservirung durchsichtiger Embryonen zum Studium der Skeletbildung. Verhandlungen der Anatomischen Gesellschaft, Bd. 13, 1897.

2. **Mall, Franklin P.**
'06 On Ossification Centers in Human Embryos less than one hundred Days Old. Amer. Jour. of Anat., Vol. V., No. 4, pp. 431–458 with 6 text figures and 7 tables. 1906.

3. **Hill, Eben C.**
'06 On the Schultze clearing method as used in the anatomical Laboratories of the Johns Hopkins University. Bull. of the Johns Hopkins Hospital, Vol. XVII., No. 181, pp. 111–115, 1906.

4. **Siebenrock, Friedrich.**
'94 Das Skelet der Lacerta Simonyi Steind. und der Lacertiden Familie überhaupt. Sitz. der Kaiserl. Akad. der Wissenschaften in Wien. Mathem.-Naturw. Classe, Bd. CIII., Abth. I., April, 1894.

5. **Gegenbaur, C.**
'71 Beiträge zur Kenntniss des Beckens der Vögel. Jenaische Zeitschrift., Bd. VI., 1871.

6. **Hoffman, C. K.**
'77–'78 Beiträge zur vergleichende Anatomie der Wirbelthiere — IX. Zur Morphologie der Rippen. Niederl. Archiv für Zoölogie, Bd. IV., 1877–1878.

7. **Siebenrock, Friederich.**
'92 Ueber Wirbel-Assimilation bei den Sauriern. Annalen der K.K. Hofsmuseums, Bd. VII., 1892.

8. **Cope, E. D.**
'92 The Osteology of the Lacertilia. Proc. of the Amer. Philos. Soc., Vol. XXX., p. 207, May 10, 1892.

9. **Huxley, T. H.**
'92 A Manual of the Anatomy of the vertebrated Animals, p. 192, New York, 1892.

10. **Parker and Haswell.**
'97 Text-Book of Zoölogy, Vol. II., p. 295, London, 1897.

Parker, J. Jeffery.
'84 A Course of Instruction in Zoötomy, p. 134, London, 1884.

Thompson, J. Arthur.
'99 Outlines of Zoölogy, pp. 570, Edinburgh and London, 1899.

Pouchet, G., et Bauregard, H.
'89 Traite d'Osteologie Comparee, pp. 355, Paris, 1889.

Zittel, Karl A.
'87–'90 Handbuch der Paleontologie. Abth. I., Bd. III., Vertebrata, p. 605. München & Leipzig, 1887–1890.

11. **Gadow, Hans.**
'01 Amphibia and Reptiles, pp. 496, London, 1901.

12. **Siebenrock, Friedrich.**
'95 Das Skelet der Agamidæ. Sitz. der Kaiserl. Akad. der Wissenschaften in Wien. Mathem. Naturw. Classe, Band CIV., Abth. I., p. 66, Nov., 1895.

13. **Gegenbaur, Carl.**
'98 Vergleichende Anatomie der Wirbelthiere mit Berücksichtigung der Wirbellosen. Bd. I., p. 251, Leipzig, 1898.

14. **Hatcher, J. B.**
'03 Osteology of Haplocanthosaurus. Mem. Carnegie Mus., Vol. 2, No. 1, pp. 14–22, 1903.

15. **McGregor, J. H.**
'06 The Phytosauria, with especial reference to Mystriosuchus and Rhytidodon. Mem. of the Am. Mus. of Nat. Hist., Vol. IX., Pt. II., p. 69, 1906.

16. **Zittel, Karl A.**
'87–'90 Handbuch der Paleontologie. Abth. I., Bd. III., Vertebrata, p. 606, München & Leipzig, 1887–1890.

17. **Broom, R.**
'03 On the Skull of a true Lizard (*Paliguana Whitei*) from the Triassic Beds of South Africa. Records of the Albany Museum, Vol. I., No. 1, p. 1, 1903.

18. **Marsh, O. C.**
'92 Notice of new Reptiles from the Laramie Formation. Am. Jour. of Sci., Vol. XLIII., p. 450, May, 1892.

19. **Williston, S. W.**
'04 The Relations and Habits of the Mosasaurs. Jour. of Geol., Vol. XII., No. 1, p. 51, 1904.

20. **Woodward, A. S.**
'98 Vertebrate Paleontology, pp. 190, Cambridge, 1898.

21. **Seeley, H. G.**
'81 On Remains of a Lizard, Q. J. G. S., Vol. 37, pp. 52–56, 1881.

22. **von Meyer, H.**
'60 Paleontographica. Bd. VII., p. 223, Pl. XXIV., 1860.

23. **Owen, R.**
'51 Fossil Reptilia of the Cretaceous Formations, p. 28, Pl. X., London, 1851.

24. **Kramberger, Gorjanovic-Carl.**
'92 Aigialosaurus, eine neue Eidechse aus der Kreideschiefern der Insel Lesina. Societas Historico-Naturalis Groatica, p. 20, 1892.

25. **Kornhuber.**
'01 Abhandlungen der k. k. Geol. Reichsanstalt, Bd. XVII., Heft 5, pp. 1–24 Pls. I.–III., 1901.

SPERMATOGENESIS IN PHILOSAMIA CYNTHIA.

PAULINE H. DEDERER.

An investigation of spermatogenesis in *Philosamia cynthia*, a moth of the Saturnid family, was undertaken in the summer of 1905, at the suggestion of Professor Crampton, who for some years has been engaged in a statistical study of variation in this form.

Few researches have as yet been made in the spermatogenesis of Lepidoptera. Platner, in a paper published in 1886, appears to have been the first to describe the development of germ cells in this group of insects. Here, however, in his plates of *Pygæra* and *Sphinx*, he gave no details of chromosome numbers and divisions, but figured chiefly the development of cytoplasmic structures in the spermatid. Among other writers who have concerned themselves principally with the cytoplasmic aspect of development, may be mentioned Meves ('97) and La Valette ('97). Munson ('06) figures a few chromosome groups in connection with an extensive account of the development of achromatic structures in the spermatogenesis of *Papilio*. Toyama's paper on the silkworm I have not been able to obtain. The observations of Miss Stevens, published in the past year, upon the spermatocytes of the butterflies *Cacœcia* and *Euvanessa*, will be referred to later.

MATERIAL.

The life history of *Philosamia* is, briefly as follows: The eggs are laid the early part of June; develop into larvæ which pupate in September, and remain in the pupal stage until their emergence as moths the following June. The development of the spermatocytes takes place in the pupa. The testes are kidney-shaped bodies, about one eighth inch long, lying within the body cavity directly beneath the abdominal tergum. They are enveloped by voluminous yellow fat bodies, from which they can be readily distinguished by their lighter color, and compact shape. The testis (Fig. 1) is divided into four lobes, by three thin sheets of

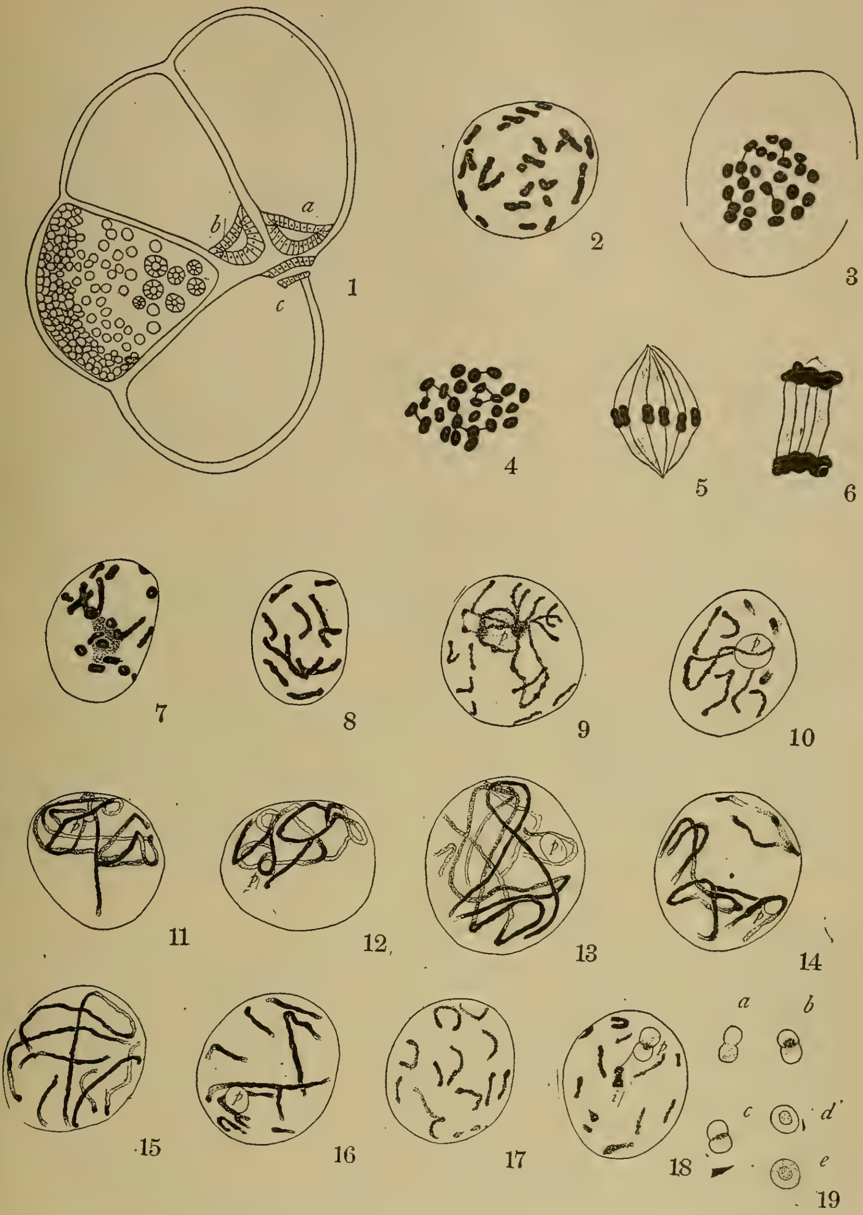


Fig. 1. Diagram of horizontal section through testis (enlarged 20 diameters) showing arrangement of cysts in the lobes. Openings of vas deferens cut obliquely at *a* and *b*, longitudinally at *c*.

Fig. 2. Early prophase of last spermatogonial division.

Figs. 3, 4. Polar view of last spermatogonial metaphase.

Figs. 5, 6. Side views of metaphase and of telophase (optical sections).

Figs. 7–10. Various stages in spireme formation; appearance of plasmosome.

Figs. 11, 12. Concentrated stage of spireme.

Fig. 13. Height of spireme stage.

Figs. 14–16. Stages in breaking up of spireme.

Fig. 19, *a–e*. Various views of plasmosome from nuclei similar to Fig. 18.

connective tissue, which extend from the outer or convex side of the organ, and converge towards the "hilus," where each lobe opens into the sperm duct. The mature cells lie near this point. Spermatogonia are massed closely together at the opposite end, and the cells in the growth stage are grouped together in rounded cysts which lie free in the lumen of the lobe.

METHODS.

Upon removal of the dorsal abdominal wall, the testes were quickly dissected out and transferred immediately to the fixing fluid. Corrosive-acetic, Gilson's alcohol-chloroform-acetic, and Flemming's fluids were used. The two former fixing agents proved especially good for spireme stages, but achromatic structures were more clearly defined with Flemming's fluid. The sections were stained with iron hæmatoxylin, with which it was possible to differentiate the plasmosome, or true nucleolus, from the chromatic nucleolus. Thionin was also used, but did not differentiate so clearly.

The figures for this paper are, with the exception of Fig. 1, from camera drawings made with compensating ocular No. 8, with a tube length of 160 mm., and $\frac{1}{12}$ oil immersion lens. They were enlarged $2\frac{1}{2}$ diameters with a drawing camera, corrected from the original, and then reduced one half in the final plates.

GENERAL DEVELOPMENT.

Owing to the long period of development, it is not possible to find all stages of germ cells in any one testis. In material fixed during the winter, the series ranges from spermatogonia to perhaps only the first spermatocyte prophase, while in testes fixed in early June, about one week before emergence, nearly all of the cells are transformed into spermatids and spermatozoa.

There are several interesting points of general development to be observed in the winter material. A varying amount of disintegration takes place in the cells. A few first divisions appear in the autumn pupæ, but at a later period none are found, so that it is probable that these are precocious first divisions, which are followed by disintegration, while the permanent division stages appear in the spring. This observation differs from that of Wilcox, who found in *Caloptenus* that "if cells reach the spermatocyte stage they complete their course."

The cells in the growth stage also show a certain amount of disintegration, when the chromatin appears to be concentrated into one or more spherical bodies resembling yolk granules. Another point observed is that the spermatogonia along the periphery proliferate inwards a new growth at one point, forming a compact mass, the later development of which may go to make up the deficiency caused by disintegration.

In several preparations of very late stages, made in the early summer, where nearly all the cells were in the spermatid phase, small groups of giant spermatogonia were observed near the periphery of the testes. The stages ranged only from prophases to anaphases. Metaphase groups were most frequent, and several clear counts were obtained giving twenty-six chromosomes, the normal spermatogonial number. Montgomery found a similar condition in the spermatogenesis of *Peripatus*. All these giant spermatogonia were in mitosis, *i. e.*, from late prophase to anaphase. No earlier nor later stages were observed. Montgomery found that these cells were more numerous in cysts showing disintegration, and concluded that they were "hypertrophied spermatogonia, whose mitosis proceeds normally as far as the anaphase, when atrophy begins." In my preparations I have not observed that the presence of giant spermatogonia is correlated with disintegration in the cysts.

SPERMATOGONIA.

The chromosomes of the last spermatogonial metaphase can be quite clearly seen in polar view, connected in many cases by what appear to be thin black threads (Figs. 3, 4). They are rounded bead-like bodies of approximately the same size. When turned obliquely they show a bipartite form, preparatory to the last spermatogonial division, and consequently appear larger. In the best polar views of the metaphase, twenty-six chromosomes can be seen. Only in cases where the chromatin elements are more concentrated, and hence difficult to count, does the number appear less. A side view of the equatorial plate, an optical section (Fig. 5), shows six bipartite chromosomes, arranged in a regular line at the center of the spindle. Many side views with a much larger number were seen, but this is typical of the regularity of form and division of all the chromosomes. The nuclear membrane disappears at the metaphase. In telophase, the chromosomes are crowded into a dense mass, in which it is difficult to determine the outlines of individual ones (Fig. 6).

After the daughter cells have formed, and the nuclear membrane again encloses the chromosomes, the nucleus increases in size, and the chromosomes spread out into the cavity. Figs. 7, 8 show a transition from the characteristic round or oval chromosomes of the spermatogonia into rods of varying length and

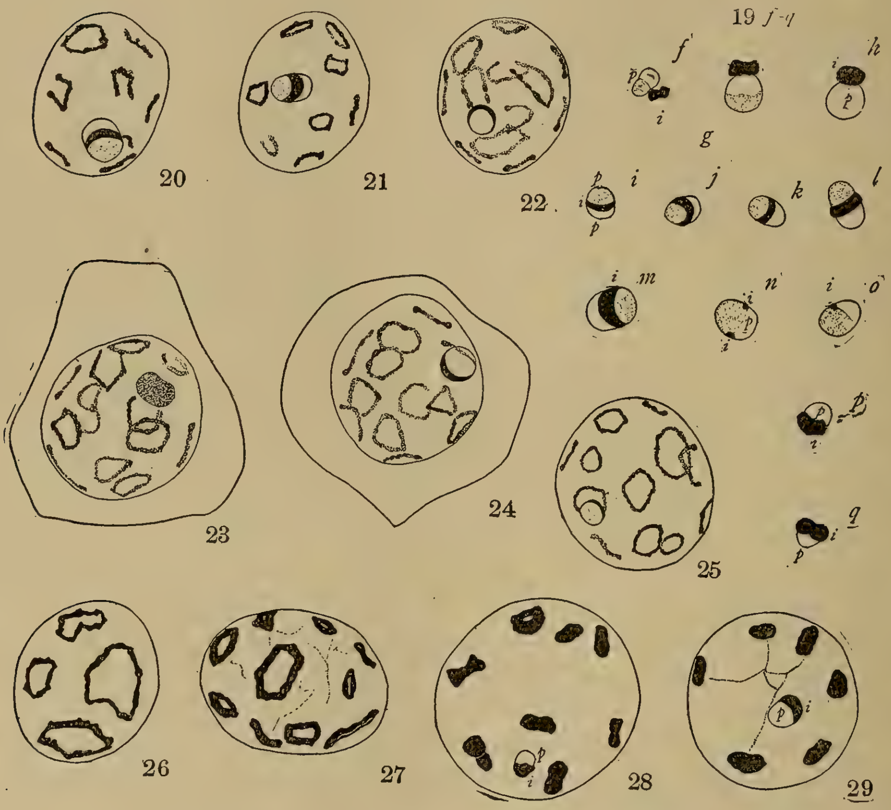


FIG. 19, *f–q*. Various views of nucleoli during growth period, showing relation of idiochromosome to plasmosome.

FIGS. 20, 21. Early ring stage—idiochromosome attached to plasmosome.

FIGS. 22–25. Four views of later stage—showing twelve rings and nucleolus.

FIGS. 26–29. Contraction of rings into chromosomes.

uneven contour. Several of the chromosomes are grouped around a dark gray mass near the center of the nucleus, in which I believe, from what occurs later, the plasmosome appears.

The chromosomes seem to transform from rods, into longer and thinner skein-like pieces, with rougher outline, and lighter staining capacity. In Fig. 9 some of these pieces are seen to form a spireme, and this, like the chromosomes in the preceding figure, is centered around a large gray-staining mass, which is seen in Fig. 10 to be a definite plasmosome.

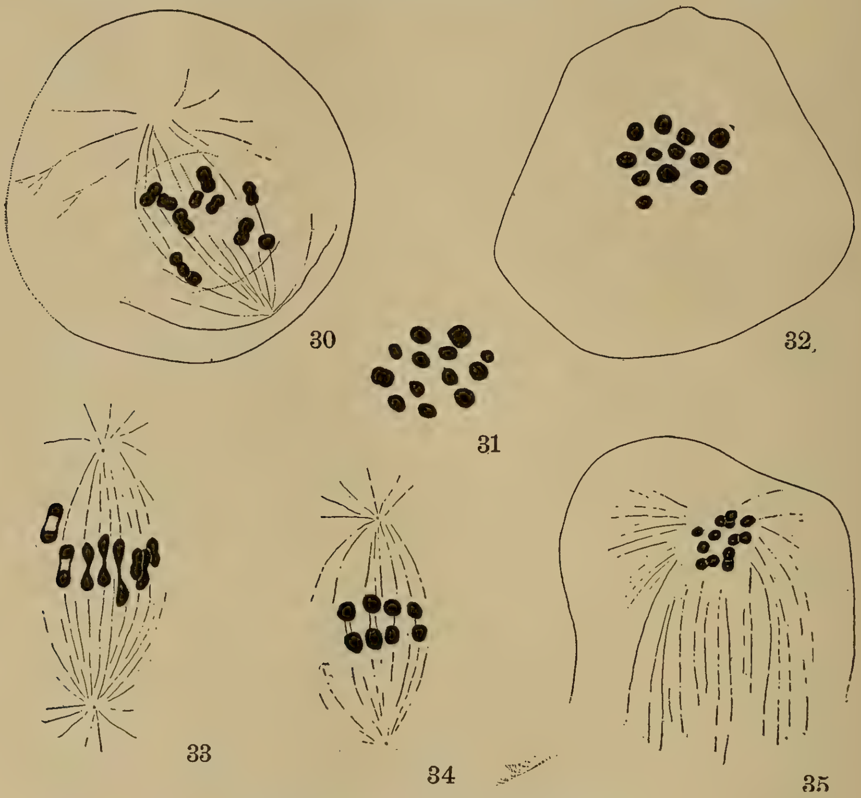
Spireme Stage.

Figures 11, 12 are characteristic of the next definite stage. The chromatin pieces now form a close intricately-coiled spireme, the mass being contracted against one side of the nucleus, nearest the greatest amount of cytoplasm, as Montgomery described in *Syrbula*. The spireme is not continuous, for several free ends are seen in sections which give a complete focus of the nuclei; but it is impossible to determine the number of threads which form it. A plasmosome is seen, entangled in the meshes of the spireme. The threads are thicker and smoother in outline than in Fig. 9, and stain black even in sections which in other respects are light in color.

The question of synapsis was carefully studied in stages from 7 to 11, but I have not been able to obtain decisive evidence regarding the nature of the process. I was unable to find evidence that the chromatin rods unite definitely two by two in forming the spireme, but that they do unite in some manner seems clear. Nowhere have I found stages which might be interpreted as parasynapsis, or side by side union of the chromosomes, such as has been figured by the Schreiners, and other observers, nor could I discover that in the spireme the threads show a longitudinal split.

After contraction at one side of the nucleus, as shown in Figs. 11, 12, the spireme spreads out to occupy the entire nuclear cavity, which increases in size. Fig. 13 represents an unusually large nucleus, where the coiling of the threads, and the plasmosome, are distinctly seen.

We may speak of this condition as the height of the spireme stage. From this point onward, the spireme threads appear to break up into shorter elements, and to become somewhat more disentangled from one another, as figured in Nos. 14 and 15. In the latter the plasmosome is not in the plane of section. In Fig. 16 this fragmentation has become more marked, until in Fig. 17, the spireme is transformed into thirteen pieces, of irregular shape and outline, which are more granular and stain less intensely, than the previous stages. (The plasmosome does not appear in this section.) In Fig. 18 the limit of spireme fragmentation has been reached. Thirteen chromatin elements appear, one of which is denser than the others. The plasmosome is characteristically bipartite in this and later stages.



FIGS. 30–35. First spermatocyte division.
FIG. 30. Prophase (two chromosomes lacking in this section).
FIGS. 31, 32. Polar views of metaphase groups showing 13 chromosomes.
FIGS. 33, 34. Side views of anaphase (not all the chromosomes are shown).
FIG. 35. Telophase.

There is a very constant difference in the appearance of the two parts of the plasmosome. One half is clear and transparent, the other, slightly granular, and stains a deeper gray. Various views of it are shown in Fig. 19, *a* to *e*, drawn from nuclei of the same stage as Fig. 18. Three side views are given in Fig. 19, *a–c* ; in the two latter the gray half seems more darkly granular at its region of union with the other. In end view, *d*, *e*, only one part is seen in outline, so that the structure appears as a single sphere, but a smaller granular area is found in the center, which seems to be nothing but the granular region indicated in *b*, seen through the clear half of the plasmosome.

The intervening history from the breaking up of the spireme into thirteen elements, and the appearance of a double plasmosome, up through the stage when rings are formed in the growth period, will be passed over for the present, to a consideration of the

Maturation Divisions.

In prophase of the first maturation division, the chromosomes appear regularly bipartite, and approximately equal in size (Fig. 30), placed irregularly upon the spindle, in preparation for metaphase. (The section lacks two of the typical number.) Many very clear metaphase groups, seen in polar view, show invariably thirteen chromosomes. Two incomplete sections (Figs. 33, 34) give views of typical anaphases, where division always appears equal.

Polar views of the second metaphase (Figs. 36, 37) have the same grouping and appearance as in the first division, the only difference being in the size of the chromosomes. In a late anaphase of second division (Fig. 38), the chromosomes of each group are approximately similar in size, and in none of the anaphases studied have I seen a case of unequal division. In early telophase the chromosomes are crowded together, and difficult to count, but several counts from polar view, or slightly oblique, showed the usual number, thirteen. Figures 39–41 are various views, all showing thirteen chromosomes as a result of second division. Fig. 42 shows the characteristic lengthening of the spindle in this division.

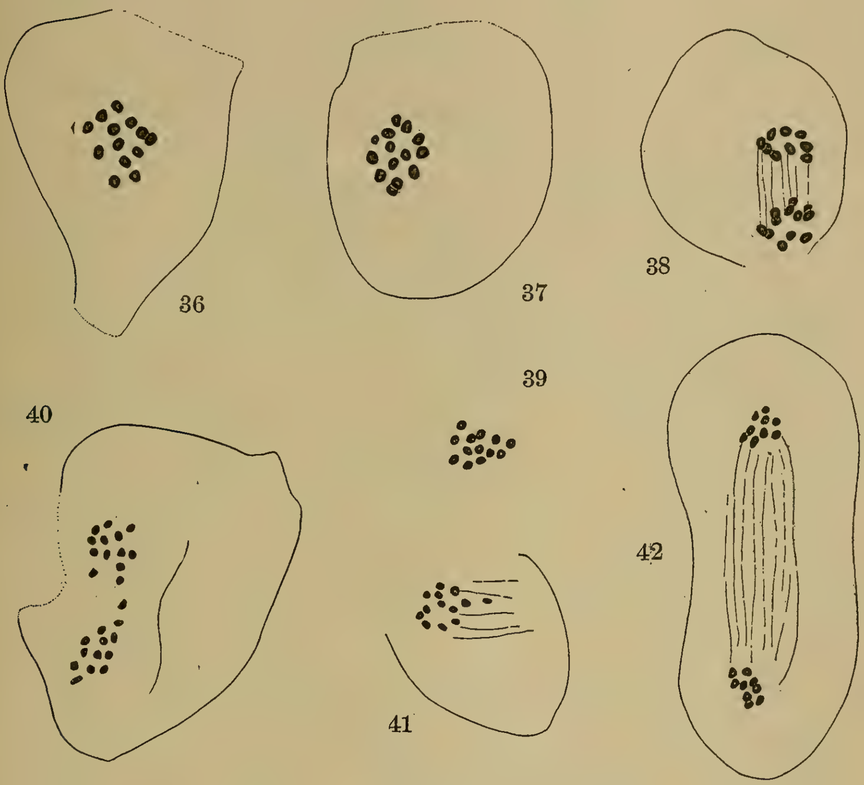
From the foregoing facts it is clear that in *Philosamia* there is no odd or "accessory" chromosome, and since there seems to be also no unequal division of chromatin material, there is no element that can be distinguished as an idiochromosome-pair in the metaphase. There is, however, reason to believe that one of the bivalents differs from the others during the growth period in such a way as to indicate that it is to be identified as an equal pair of idiochromosomes, comparable with that described by Wilson in the case of *Nezara*.

Ring Stage.

Having found twenty-six chromosomes in the spermatogonia. thirteen broken chromatin elements after the spireme stage (Figs. 17, 18), and thirteen chromosomes in maturation metaphases, I naturally expected to find that in the ring stage resulting after breaking of the spireme, thirteen rings were present. I found, however, only twelve, in a very large number of cells which I examined (Figs. 22–25). No clear case showing thirteen was observed.

My next step was to study the nucleolus present in the ring stage, to see if it might be different from the earlier double plasmosome, in containing some chromatin material. I found the double plasmosome, previously noted in Figs. 18 and 19, *a–e*, and in addition a deep black crescent-shaped band attached around the middle, its concave edge directed always toward the darker portion of the plasmosome as in Figs. 20 and 21, and 19, *m*, *n*, *o*, the two latter being views of the reverse side of the nucleolus, showing that the chromatin does not completely encircle the plasmosome. This chromatin band I interpret as equivalent to the two equal idiochromosomes found by Wilson in *Nezara*, the band thus being bivalent, as is each of the rings, and the number of chromatin elements thus forming the correct reduced number thirteen.

How, and when, does this chromatin band become associated with the plasmosome? The evidence seems to show that it is derived from one of the chromatic elements — in the case of Fig. 18, probably the darker, broader mass — and attaches itself at this stage to the plasmosome. In Fig. 19, *f*, is represented a plasmosome with the chromatin mass drawn up near it, preparatory to attachment. The other chromatin elements in this nucleus are all irregular and feathery in outline, as in Fig. 18. Fig 19, *g*, *h*, is an end view of the same stage, showing a larger structure, with the same characteristic chromatin mass. In *i* and *j* this has become attached to the plasmosome. From the broken chromatin rods the rings are formed, apparently by a bending around of the rods; but the exact manner of the change seems very difficult to determine (Figs. 20, 21). In this stage, which lasts throughout the winter, the double nucleolus with its chromatin band, is a constant factor. The rings are irregular in size and



FIGS. 36–42. Second Spermatocyte Division.
FIGS. 36, 37. Polar views of metaphase groups with 13 chromosomes.
FIG. 38. Anaphase.
FIG. 39. Polar view of telophase.
FIGS. 40, 41. Late anaphase groups, side view, showing 13 chromosomes.
FIG. 42. Telophase (not all the chromosomes appear).

form, very granular in appearance, and stain less deeply than the spireme.

Concentration of Rings.

This is the next well-marked stage. The threads forming the rings become thicker, rougher in outline, and more deeply staining (Fig. 26), and as they thicken their circumference decreases until the originally large central space is reduced to a minute cavity, which is finally closed up altogether, and a chromosome results. Fig. 27 shows various stages in concentration. Chromosomes as shown in Fig. 28 and 29 succeed this until the at first irregular elliptical masses have assumed the smooth dumb-bell-like form seen in the first prophase stage (Fig. 30).

During concentration of the rings, the idiochromosome also appears to thicken, becoming shorter and broader, so that it has a smaller surface of contact with the plasmosome (Fig. 19, *p*, and 28). In Fig. 19, *q*, from an early prophase, it has the smooth bipartite form common to the other chromosomes. From this time, the plasmosome disappears, and the idiochromosome is indistinguishable from the others.

Conclusion.

The presence of a double idiochromosome in *Philosamia* connects it in this respect, with *Euvanessa* and *Cacœcia*, two species of butterflies studied by Miss Stevens ('06), who finds an equal pair of idiochromosomes, or "*sometimes a two lobed body*," . . . "whose only apparent peculiarity is its condensed form during growth."

In the case of the moth the idiochromosome appears single from the time of its first appearance, but it would seem that it is a *bivalent* body, in just the same way that the rings and resulting chromosomes are bivalent. This bivalence has its origin in all probability, in the prespireme stage.

Philosamia thus lies at the opposite end of a series, from *Nezara*, where the equal idiochromosomes do not unite until after the first division. An intermediate stage is represented in *Brochymena* (Wilson, '05), where the idiochromosomes, in this case unequal, lie at first separated, but later united, in the growth period. Wilson concludes for this form that, "when only one chromatin nucleolus is present, it is to be considered as a bivalent body, arising by fusion or synapsis of the two idiochromosomes." In *Brochymena,* however, they separate again before the first division.

I wish to acknowledge my indebtedness to Professor Crampton for suggestions and material and also to Professor Wilson for kindly supervision and corrections, and reading of manuscript.

SUMMARY.

1. The spermatogonia contain twenty-six chromosomes, of approximately the same size and shape.

2. There is a definite spireme stage with a simple plasmosome.

3. The spireme segments into thirteen parts, of which twelve form rings, the thirteenth becoming attached as a chromatin mass to the plasmosome, which at this stage is double.

4. In the growth period, when the twelve rings are definitely formed, the chromatin mass is bent in a crescentic band around the plasmosome, forming a chromosome nucleolus.

5. This band represents a pair of idiochromosomes, and is bivalent like the rings, but always appears as a single body.

6. First and second metaphases show thirteen chromosomes. Divisions are equal, so that the spermatids contain similar chromosome groups.

COLUMBIA UNIVERSITY,
March, 1907.

LITERATURE.

La Valette St. George

'97 Zur Samen- und Eibildung beim Seiden-spinner (Bombyx mori), Arch. Mikr. Anat., 50.

Meves, F.

'07 Über Centralkörper in männlichen Geschlechtszellen von Schmetterlingen. Anat. Anzeig., 14.

Montgomery, T. H.

'00 The Spermatogenesis of Peripatus (Peripatopsis) Balfouri up to the Formation of the Spermatid. Zool. Jahrb., 14.

'05 The Spermatogenesis of Syrbula and Lycosa, with general considerations upon Chromosome Reduction and the Heterochromosomes. Proc. Acad. Nat. Sci. Phil., Feb., 1905.

Munson, J. P.

'06 Spermatogenesis of the Butterfly, Papilio Rutulus. Proc. Boston Soc. Nat. Hist., 33, no. 3.

Platner, G.

'89 Beiträge zur Kenntniss der Zelle und ihrer Theilung. V. Samenbildung. und Zelltheilung in Hoden der Schmetterlinge. Arch. Mikr. Anat., 33.

Schreiner, A. and K.

'06 Über Chromatin-reifung der Geschlechtszellen. Extr. Archives de Biol., 22.

Stevens, N. M.

'06 Studies in Spermatogenesis. Part II. A comparative study of the Heterochromosomes in certain species of Coleoptera, Hemiptera and Lepidoptera, with especial reference to Sex Determination. Carnegie Inst. of Washington, Pub. no. 36.

Wilcox, E. V.

'95 Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen. Bull. Mus. Comp. Zool. Harvard, 27.

Wilson, E. B.

'05 Studies on Chromosomes. I. The Behaviour of the Idiochromosomes in Hemiptera. Jour. Exp. Zool., 2, no. 3.

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

VOL. XIII AUGUST, 1907 No. 3

CONTENTS

		PAGE
ELLIS, MAX M.	*The Influence of the Amount of Injury upon the Rate and Amount of Regeneration in Mancasellus macrourus (Garman)*	107
MCCLENDON, J. F.	*The Spermatogenesis of Pandarus sinuatus Say*	114
PATTERSON, J. THOS.	*The Order of Appearance of the Anterior Somites in the Chick*	121
FIELDE, ADELE M.	*Suggested Explanations of Certain Phenomena in the Lives of Ants; with a Method of Tracing Ants to their Respective Communities*	134
CHILD, C. M.	*Studies on the Relation between Amitosis and Mitosis, III*	138
HEATH, HAROLD.	*The Longevity of Members of the Different Castes of Termopsis angusticollis*	161

Smithsonian Institution
National Museum

PUBLISHED MONTHLY BY THE

MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY

THE NEW ERA PRINTING COMPANY

LANCASTER, PA.

AGENT FOR GREAT BRITAIN	AGENT FOR GERMANY	AGENT FOR FRANCE
WILLIAM WESLEY & SON	R. FRIEDLÄNDER & SOHN	LIBRAIRIE ALBERT SCHULZ
28 Essex Street, Strand London, W. C.	*Berlin, N. W. Carlstrasse, 11*	*3 Place de la Sorbonne Paris, France*

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter,
under Act of Congress of July 16, 1894.

Editorial Staff.

E. G. CONKLIN—*The University of Pennsylvania.*

JACQUES LOEB—*The University of California.*

T. H. MORGAN—*Columbia University.*

W. M. WHEELER—*American Museum of Natural History, New York.*

C. O. WHITMAN—*The University of Chicago.*

E. B. WILSON—*Columbia University.*

Managing Editor.

FRANK R. LILLIE—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Holl, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa.

Vol. XIII. *August, 1907.* *No. 3.*

BIOLOGICAL BULLETIN

THE INFLUENCE OF THE AMOUNT OF INJURY UPON THE RATE AND AMOUNT OF REGENERATION IN MANCASELLUS MACROURUS (GARMAN).[1]

MAX M. ELLIS.

I. Introduction.

Until Dr. Zeleny announced the results of his experiments on the fiddler crab, *Gelasimus*, and the brittle star, *Ophioglypha*, it was generally thought that an increase in the amount of injury would decrease the ability to repair injury, that is, it would retard regeneration. In these two forms he found that an increase in the degree of injury produced a corresponding increase in the rate of regeneration. Later he established the same principle as true for the common crawfish,[2] *Cambarus propinquus.*

From the crawfish experiment he concludes that "in series with the greater degree of injury each chela regenerates more rapidly than the single removed chela of the series with the lesser degree of injury." These three experiments present the idea that an increase in the amount of injury accelerates rather than retards regeneration. However, it is probable, as is indicated by Dr. Zeleny's work on the brittle star, that there is a degree of injury, a limit, beyond which this is not the case. In the work just mentioned,[3] when all five of the arms were removed the regeneration was slower than when four were removed.

The present experiments were made with the object of contributing some quantitative data concerning the relation of the amount of injury to the rate and amount of regeneration. The

[1] Contribution from the Zoological Laboratory of Indiana University. No. 85.

[2] *Jour. Ex. Zoöl.*, Vol. II., No. 3, '05.

[3] *Jour. Ex. Zoöl.*, Vol. II., No. 1.

form *Mancasellus* was chosen because it is abundant in this region. It regenerates lost parts readily and as each leg has a breaking joint at the coxal-thoracic articulation it is possible to make all operations uniform. The results obtained are, in a measure, parallel with Dr. Zeleny's work.

II. METHODS.

The isopods used in these experiments were taken in a stream near the Indiana University campus at Bloomington, from a part not over three hundred feet in length. In this distance it is probable that they had been subjected to the same general conditions previous to the experiments.

Set I. — For Set I. several hundred specimens were collected on October 3, 1906, from which twenty normal males measuring between 10–13 mm. were selected. These were divided into four series. In series A a "standard" injury was established by the removal of the right sixth walking leg. In each of the remaining three series the operation was the infliction of the "standard" injury plus an "added" injury. As "added" injury the right fifth walking leg was removed in series B, the right fifth, fourth and third in series C and all of the right thoracic appendages including the cheliped in series D. The operation was made in each series by pinching the tip of the appendage to be removed till the animal cast it off at the breaking joint. The above system was repeated daily on a fresh catch of Isopods for five days, that is, until October 8, 1906, when the completed set consisted of four series of twenty-five individuals each. Each specimen was kept in a twelve-ounce saltmouth bottle, which was inclined a few degrees from the horizontal by the mouth resting on an inch block. The water was changed every six days. As food, the partially decayed leaves of the common elm were supplied. An excess of leaves was always present. Fourteen days after the date of operation each individual was killed.

Set. II. — The second set, series E, F and G, was planned differently in order to obtain a uniform relation between the time of the last moult and the date of operation. On January 24, 1907, several hundred isopods were collected and two hundred and fifty normal males measuring between 13–16 mm. selected. These were placed in individual bottles at once. Twenty-four hours later they were examined and twenty found to have moulted. These twenty were isolated for another twenty-four hours. At the end of the second day, that is, not more than forty-eight hours after their last moult, twelve individuals of about the same size from this twenty were operated upon. Thus a double check was made on the size of the specimens for only those whose length, both before and after the moult was about he same, were retained. Three series were used. In series E the right sixth walking leg was removed to again establish a "standard" series.

In the other two series "added" injury was also inflicted. The removal of right fifth and fourth walking legs constituted this "added" injury in series F and all right walking legs in series G. In the above manner twenty-one individuals were selected and operated upon on the twenty-sixth, nine on the twenty-seventh and eighteen on the twenty-eighth, giving a completed series of sixty individuals, twenty to a series. These series were maintained in the same manner as Set I., save that the water was changed daily. The method of operation was also the same. Two days after their next moult the isopods were killed. By February 11, 1907, twenty-eight had moulted and the set was discontinued.

III. Data.

The length in millimeters of the body, the original leg and the regenerated leg is given for each individual that had regenerated at the close of the experiment. The specific amount of regeneration, which is the per cent. of regeneration in terms of the original leg is given for both sets. The specific rate, which is the specific amount divided by the number of days in the moulting period was obtainable only for Set II.

Explanation of Tables.[1]

Set I. Series A. Right sixth walking leg removed.
Series B. Right 6–5 walking legs removed.
Series C. Right 6–5–4–3 walking legs removed.
Series D. Right 6–5–4–3–2–1 walking legs and cheliped removed.
Set II. Series E. Right sixth walking leg removed.
Serses F. Right 6–5–4 walking legs removed.
Series G. Right 6–5–4–3–2–1 walking legs removed.

---

[1] Abbreviations: Orig., original; Reg., regenerated; Spec. Amt., specific amount; Spec. Rate, specific rate; 13 +, moulted during last half of thirteenth day.

TABLE I.

SET I. *Series A.*

Cat. No.	Body Length.	Right Sixth Walking Leg.		Spec. Amt.	Moulting Period—Days.	
		Orig.	Reg		1st.	2d.
1	9	3.48	2.52	.72	13	
2	9	4.00	3.19	.80	2	
3	10	3.76	2.82	.76	13	
4	10	3.57	2.48	.69	12	
5	10	4.00	2.71	.67	13	
6	10	3.38	2.90	.86	13+	
7	10	3.76	2.52	.67	7	11
8	10	4.33	2.81	.65	13	
9	11	3.38	1.81	.54	12	
10	11	5.33	2.38	.45	13	
11	12	4.66	3.19	.69	13+	
Av.	10.1			.68		

Series B.

1	10	4.14	2.86	.69	13	
2	10	3.43	2.76	.80	11	
3	10	3.76	2.71	.72	9	
4	11	3.43	2.76	.80	1	8
5	11	4.29	2.19	.51	13+	
6	11	3.67	2.38	.68	8	
7	11	3.52	3.29	.93	4	
8	11	4.05	2.57	.63	13+	
9	11	3.19	2.57	.81	8	
10	11	4.38	2.76	.63	13	
11	12	3.67	3.43	.93	13	
Av.	10.8			.739		

TABLE II.

SET I. *Series C.*

Cat. No.	Body Length.	Right Sixth Walking Leg.		Spec. Amt.	Moulting Period—Days.	
		Orig.	Reg.		1st.	2d.
1	10	4.48	2.82	.63	13	
2	10	3.62	2.86	.79	7	13+
3	10	3.52	2.05	.58	11	
4	11	4.05	2.57	.63	2	13+
5	11	4.29	2.81	.65	13+	
6	11	4.14	2.86	.69	12	
7	12	4.00	3.10	.775	3	10
8	12	4.43	2.95	.86	9	
9	12	4.48	2.43	.54	10	
10	13	4.29	2.82	.88	2	13
Av.	11.2			.703		

Series D.

1	9	3.38	2.33	.66	10	13+
2	10	3.62	2.57	.71	13	
3	10	3.95	2.76	.69	3	
4	10	3.29	2.52	.77	12	
5	10	3.86	1.91	.49	7	
6	11	4.10	3.33	.81	8	
7	11	3.90	2.71	.72	7	
8	11	4.19	2.90	.69	12	
9	12	4.19	3.19	.76	12	
10	12	4.61	3.19	.67	11	
11	13	4.24	1.91	.45	7	
12	13	5.23	2.90	.55	13	
Av.	11.0			.664		

TABLE III.

SET II. *Series E.*

Cat. No.	Body Length.	Right Sixth Walking Leg.		Spec. Amt.	Moulting Period.	Spec. Rate.
		Orig.	Reg.			
1	15	7.25	2.90	.40	12	.033
2	14	5.86	2.38	.41	10	.041
3	14	5.76	2.71	.48	13	.037
4	13	6.00	2.57	.43	14	.031
5	13	5.76	2.71	.48	13	.037
Av.	13.8			.441	12.4	.036

Series F.

1	16	7.19	3.10	.43	17	.025
2	15	7.00	2.95	.42	16	.026
3	15	7.00	3.14	.45	15	.030
4	14	5.67	3.48	.61	14	.044
5	14	6.95	3.00	.43	16	.027
6	14	6.48	3.00	.46	16	.029
7	13	6.14	3.10	.50	17	.029
8	13	5.38	2.43	.45	13	.035
9	13	5.57	2.14	.38	16	.024
Av.	14			.48	15.5	.030

Series G.

1	16	6.48	3.10	.48	15	.032
2	15	7.00	2.57	.37	12	.031
3	15	6.67	2.71	.41	13	.031
4	15	6.24	2.95	.47	13	.036
5	14	6.90	3.14	.46	17	.027
6	14	6.00	2.62	.44	15	.029
7	14	5.33	2.33	.44	16	.028
8	14	5.86	2.67	.46	14	.033
9	14	5.05	2.76	.55	13	.042
10	14	4.95	2.90	.59	15	.045
11	14	6.48	3.10	.45	13	.035
12	14	6.67	2.90	.44	16	.028
13	14	6.43	2.95	.46	13	.044
14	14	4.76	2.29	.48	10	.048
Av.	14.3			.463	13.5	.035

IV. Results.

1. *Specific Amount of Regeneration.*

Set. I. A — .67, B — .74, C — .70, D — .66.

Starting with the standard series A the specific amount of regeneration increases in series B and decreases in series C and D. Both B and C are greater than A, while D is less than A. These values show two important facts: (1) that there is an optimum degree of injury and (2) that there is a limit to the amount of added injury that may be inflicted and the resultant regeneration still be greater than that following the standard injury. This limit of added injury is between C and D.

Set. II. E — .44, F — .48, G — .46.

The specific amounts of Set II. follow precisely the same rule of arrangement as those of Set. I. There is a rise and a fall in the amount of regeneration. However no series of Set. II. is below standard and as a result the limit of added injury does not occur. Since the injury inflicted in series A and E was the same, there being so few individuals in series E it was thought advisable to obtain the values of Set II. in terms of Set I. Accordingly a coefficient (1.542) was established by dividing the specific amount of series A by that of series E. The specific amounts of Set II. were then multiplied by this and the following table made by placing the values of both sets in the order of amount of injury.

A and E — .68, B — .74, F — .74, C — .70, G — .70, D — .66.

In this table of the combined values there is the same plan of increase and decrease in the amount of regeneration as has been noted in both sets. It is a rise at the first followed by a steady decline. The limit of added injury and the optimum are both present.

Considering these tables three things are evident:

1. The amount of regeneration increases directly with the amount of injury until an optimum has been attained.

2. Beyond the optimum added injury still gives an amount of regeneration greater than that of the standard injury up to a limit.

3. Beyond this limit regeneration is less than the standard.

2. *Specific Rate.*

The specific rates for Set I. were not obtainable as the time and not the moult was constant. For Set II. the specific rates were E — .036, F — .030, G — .035. Nothing very certain can be said as to the value of these figures, however, as specific rate may be an unreliable quantity. The greatest source of error in computing it is the moulting period. This could easily be influenced either by (1) the shock of the operation, or (2) by the asymmetrical condition produced by the loss of appendages. The effect of neither was determined, yet because of their existence as possible factors in the rate of moulting the exact worth of the specific rate is not known.

V. Conclusion.

From the data collected it seems probable that an increase in the amount of injury produces an increase in the amount of regeneration until a certain limit is reached. This limit of added injury is probably constant for the species.

As a point of interest it may be noted that the individuals collected in October regenerated about fifty per cent. more in a given time than those collected in January. It seems that the season of the year may have some influence upon regeneration.

VI. Summary.

1. Each leg of *Mancasellus* possesses a breaking joint at the coxal-thoracic articulation.
2. The season of the year may influence the ability to regenerate lost parts.
3. Increased injury increases the amount of regeneration in *Mancasellus* until the optimum is reached. From this it decreases to a limit beyond which the amount of regeneration is less than that of the standard.
4. The optimum seems to be low for *Mancasellus*.
5. The limit of added injury is relatively high.

Acknowledgment.

This work was undertaken and carried out at the suggestion of Dr. Charles Zeleny. I thank him for his willing assistance.

THE SPERMATOGENESIS OF PANDARUS SINUATUS SAY.

J. F. McCLENDON.

The spermatogenesis of the parasitic copepods of the Woods Hole region was included as a preliminary note in a previous paper of mine (McClendon, '06), *Lœmargus muricatus* Kröyer being taken as an example. The spermatogenesis of these forms is more nearly identical than the oögenesis. In fact, the greatest difference is in the number and size of the cells in the testes. The advantage in studying *Lœmargus* was the greater size and greater number of cells in the testes, but the difficulty of obtaining living material of this genus led to the substitution of *Pandarus*.

The material for the present paper was procured at Woods Hole last summer. The testes with more or less adjacent tissue were removed and fixed in Flemming's stronger fluid. Paraffine sections, 3 to 7 microns in thickness, were cut, and stained in various ways. Iron hæmatoxylin, Delafield's hæmatoxylin, saffranin, orange G, and various combinations were found valuable. Not a great deal of attention was paid to the exact form in which the cytoplasm and nuclear sap was coagulated by the fixation, but attention was directed chiefly to the chromatin of the nucleus and to those remarkable bodies described in the previous paper under the name of nutritive spheres.

The spermatogonia (Fig. 1) are nearly isodiametrical cells with large spheroid nuclei. Each nucleus contains during the rest stage two or more nucleoli (plasmosomes) and a reticulum in which chromatin granules are imbedded. During mitosis sixteen chromosomes are formed and divided (Figs. 2 and 3), one half of each chromosome going to each of the two daughter cells.

The primary spermatocytes when first formed (Fig. 4) are similar to the spermatogonia except for size. The nucleoli never grow to the size they reach in the spermatogonia, probably because the prophase of mitosis begins early in the growth of the cell and the nucleoli begin to dissolve before they have had time to grow large. The chromosomes in the early prophase (Fig. 5) are thread-like and do not show a longitudinal split as is the case in the primary oöcytes of this species. It may be that the longitudinal split is present but cannot be seen on account of the smaller size of the chromosomes, or the splitting may occur after the synapsis, at which time a division is indicated by a constriction at right angles to the plane dividing the chromosomes of each pair (see below). Another interpretation is that in the primary oöcytes the "split" represents the division between adjacent chromosomes and is therefore after the synapsis. The chromosomes are at first sixteen in number, but as they become denser some appear to be joined end to end (Fig. 6), and by this time the nucleoli have entirely dissolved. The chromosomes collect together in a dense mass so that only their ends sticking out can be distinguished separately (Fig. 7), and after the elements of this mass separate they are seen to be eight double chromosomes united end to end (Fig. 8). The chromosomes now shorten, at the same time becoming thicker (Fig. 9), and soon a second constriction transforms each double chromosome into a tetrad (Fig. 10). Each tetrad continues to shorten until the width is as great as the length (Fig. 11). The nuclear wall dissolves and the spindle is now formed (Figs. 12, 13, 14). In the equatorial plate seven tetrads are arranged in a circle and the eighth lies in the center. It is impossible to observe whether the division is reducing or not, owing to the shape of the tetrads.

The second spermatocytic division follows immediately after the first. Each of the eight diads is divided equally between the two daughter cells (Figs. 15, 16). In the spermatids thus formed the chromosomes swell up and fuse to form nuclei, and the spermatid (Fig. 18) resembles the primary spermatocyte save for the reduction in the size and the absence of nucleoli.

By the methods used no distinction could be made out between the spermatids when first formed, but they develop into structures that show as great differences as exist between the products of the testes of any species of animals that has come to my attention. Many spermatids degenerate and appear to be absorbed as food by those remaining. Some of them elongate (Figs. 19 and 20) and begin to develop into spermatozoa. Whereas the spermatids when first formed are in groups of fours, as they elongate they collect into larger groups. The cell boundary becomes granular (Fig. 21) and the cytoplasm of adjacent spermatids fuses. Only part of this cytoplasm goes into the formation of the spermatozoa and the remainder forms a mass in the center of the group. When the spermatozoön is fully formed no distinction can be made between the cytoplasmic and nuclear parts of it in fixed preparations (Fig. 22), but it appears as a homogeneous chromatic thread tapering at each end. This spermatozoön is similar to those of barnacles and some other crustacea, and is non-motile in sea water, though it is supposed, like other crustacean spermatozoa to be stimulated to locomotion by the fluid in the ducts of the receptaculum semenis.

In some spermatids the chromatin collects into apparently homogeneous masses close to the nuclear membrane, and the nucleus grows at the expense of the cytoplasm (Fig. 23). This process continues until the cytoplasm is represented by merely a thin granular layer surrounding the distended nucleus (Fig. 24) and finally disappears entirely (Fig. 25). The nuclear sap, which is at first a thin fluid gradually becomes denser until it appears homogeneous on fixation and takes plasma stains. The time at which it acquires this power of taking stains is not sharply marked off, as it appears gradually and as it is determined by the duration of the staining and destaining process, but after all the cytoplasm has disappeared the interior of the "nucleus" is easily stained. Soon the nuclear membrane disappears and the chromatin remains adhering to the surface of the sphere of material that filled cavity of the nucleus (Fig. 26), and which was designated by the name nutritive-sphere in my former paper. The spermatozoa are often arranged with one end against one of these spheres, in a mammer similar to that in which the spermatozoa of many animals are related to the nurse cells.

When the products of the testes pass through the vasa deferentia and enter the spermatophores, the nutritive spheres form a layer next to the wall of the spermatophore, and the chromatin, which had separated as globules, forms a layer inside of the layer of spheres. Lastly the spermatozoa arrange themselves more or less radially, that is, with their ends abutting against the layer of chromatin globules. Some chromatin globules are found in the spaces between the spheres and become pressed out of shape by a pressure that transforms the nutritive spheres into polyhedrons. In spermatophores which are attached to the female, the substance of this nutritive layer is often found to have disappeared, leaving a structure resembling thin evacuated cell walls.

It would be interesting to know the chemical composition of the nutritive spheres. Heider ('79) probably supposed them to be mucilaginous, as he called them "austreibestoff." In some mosses there is found a mucilaginous substance in the antheridia, which swells in the presence of water and forces the spermatozoa out. A similar function was attributed to these spheres in Copepods by Heider, but I have found no evidence that such is the case. When the spermatophores are attached to the female, direct communication is formed with the receptaculum semenis, and there is no reason to believe that the spermatozoa could not enter the receptaculum by their own efforts. If there is a secretion in the receptaculum that would stimulate the spermatozoa into movement, it would diffuse into the spermatophore and be effective there. I have tried staining reactions on these spheres but can say only that they have a less affinity for plasma stains than the yolk spherules of most eggs, and that they are not of a fatty nature.

The position of the spermatozoa in relation to them and the fact that their substance disappears finally, led me to assume that they furnished nourishment for the spermatozoa. It has long been held that the nucleus influenced the assimilation in the cell, but so far as I know, the idea that the nucleus could form a store-house for the nourishment of other cells is new. I hesitated in putting forward such a view in my previous paper, hoping to discover a cytoplasmic origin of these spheres, but such seems not to be the case. In *Peripatus* the spermatozoa appear to draw nourishment from the nuclei of degenerated spermatids, but it is probable that such is the case in any testis in which cell degeneration occurs. In *Pelomyxa*, Goldschmidt ('06) describes the formation of "Glanzkörper" of the plasmosomes extruded from the nucleus. These "Glanzkörper" are supposed to be glycogen. In the method by which my sections were prepared, glycogen, if present, would probably be entirely washed out.

In the insect, *Ulula hyalina* (McClendon, '02), some of the eggs become modified to serve as protection to the other eggs. These abortive eggs or "repagula" develop in different ovarian tubules from the other eggs, and may receive different nourishment, but are of interest as modified sexual elements.

For comparison, each bundle of spermatids in *Pandarus* may be said to be provided with a cytoplasmic cytophore in the middle of the bundle and a greatly modified unicellular cytophore at the end of the bundle. Cytophores and basal cells are differentiated (visibly) after the second spermatocytic division (Korscheldt & Heider) in invertebrates. Nurse cells in ovaries are differentiated at an earlier stage (Marshall, '07).

BIOLOGICAL LABORATORY,
RANDOLPH-MACON COLLEGE,
ASHLAND, VIRGINIA,
March 30, 1907.

REFERENCES.

Boveri, T.
'04 Ergebnisse über die Konstitution der Chromatischen Substance des Zellkerns. Jena, 1904.

Foot and Strobell.
'07 The "Accessory Chromosome" of Anasa tristis. Biol. Bull., XII., No. 2.

Gibson, S.
'86 Etude Comparee da la spermatogenese chez les Arthropodes. La Cellule, Tome 1 and 2.

Goldschmidt
'06 Die Chromidien der Protozoen. Arch. f. Protistenkunde, 5, p. 130.

Gross, J.
'06 Die Spermatogenese von Pyrrochoris apterus L. Zool. Jahr. (Anat.), 23, No. 2.

Guthres, S.
'07 Zur Kenntnis der Heterochromosomen. Arch. Mik. Anat., 69, No. 3.

Heider, K.
'79 Die Gattung Lernanthropus. Arb. Zool. Ins. Wien, 2. Bd., 3 Hft.

Ishikawa, A.
'92 Studies in Reproductive Elements, I. Spermatogenesis, etc. in Diaptomus. Jour. Coll. Sc. Japan, Vol. 5.

Lerat, Paul.
'02 La premiere cinese de maturation — et la spermatogenese du Cyclops strenuus. Anat. Anz., 21 Bd., p. 407.

Marshall, W. S.
'07 Contributions toward the Embryology and Anatomy of Polistes pallipes, II. Zeit. wiss. Zool., 86, Hft. 2.

McClendon, J. F.

'02 Life History of Ulula hyalina. Amer. Nat., 36, p. 424.

'06 Development of Parasitic Copepods, I. Biol. Bull., XII., No. 1.

Montgomery, T. H.

'06 Chromosomes in the Spermatogenesis of Hemiptera Heteroptera. Amer. Phil. Soc., n. s., XX., 3.

Nowlin, W. N.

'06 Spermatogenesis of Coptocycla. Jour. Exper. Zool., III., No. 4.

Schreiner, A. and K. E.

'06 Neue studien über die Chromatinreifung der Geschlechtzellen. Arch. de Biologie, XXII., Nos. 3 and 4.

Schleip, W.

'06 Die Entwickelung der Chromosomem im Ei von Planaria. Zool. Jahr., 23, No. 2.

Wilson.

'05 Studies in Chromosomes 1 and 2. Jour. Exper. Zool., 2.

'06 Studies in Chromosomes 3. Jour. Exper. Zool., 3, No. 1.

Zweiger, H.

'06 Die spermatogenese von Forficula auricularia L. Jen. Zeit. f. Naturwiss., Bd. 22.

EXPLANATION OF PLATE I.

All the figures were drawn with Abbe Camera, Zeiss apochromat objective 2 mm., compensating ocular 12. They represent optical sections of varying thickness, of elements of the testes of *Pandarus sinuatus* Say.

FIG. 1. Spermatogonium. The black spheres are nucleoli.

FIG. 2. Anaphase of the last spermatogonial mitosis, seven of the divided chromosomes are shown.

FIG. 3. Telophase of the last spermatogonial mitosis.

Fig. 4. Resting stage of primary spermatocyte. The two black spheres are nucleoli.

FIG. 5. Presynapsis stage of the primary spermatocyte. The chromosomes are in the form of threads and are sixteen in number, but not all are shown in the figure.

FIG. 6. Commencement of synapsis. The chromosomes are denser than in the preceding figure.

FIG. 7. Synapsis stage. The chromosomes are so close together that they cannot be counted.

FIG. 8. Post-synapsis stage. The chromosomes have paired to form eight bivalent elements.

FIG. 9. Prophase of first spermatocytic mitosis. The chromosomes have shortened.

FIG. 10. Later prophase. The bivalent chromosomes are transformed into tetrads by a longitudinal furrow.

FIG. 11. Late prophase. The tetrads have become still more shortened.

FIG. 12. Metaphase of first spermatocytic mitosis.

FIG. 13. Equatorial plate of first spermatocytic mitosis. The eight tetrads are shown.

FIG. 14. Telophase of first spermatocytic mitosis.

FIG. 15. Metaphase of second spermatocytic mitosis.

FIG. 16. Telophase of second spermatocytic mitosis.

FIG. 17. Late telophase of same.

FIG. 18. Spermatid.

FIGS. 19–22. Stages in elongation of the spermatid to form the spermatozoön.

In Fig. 21 some of the cytoplasm is being lost and the cell boundary is granular.

In Fig. 22, which represents the spermatozoön, the elongated nucleus and its thin covering of cytoplasm cannot be separately distinguished.

FIGS. 23–26 represent stages in the formation of a nutritive sphere from a spermatid.

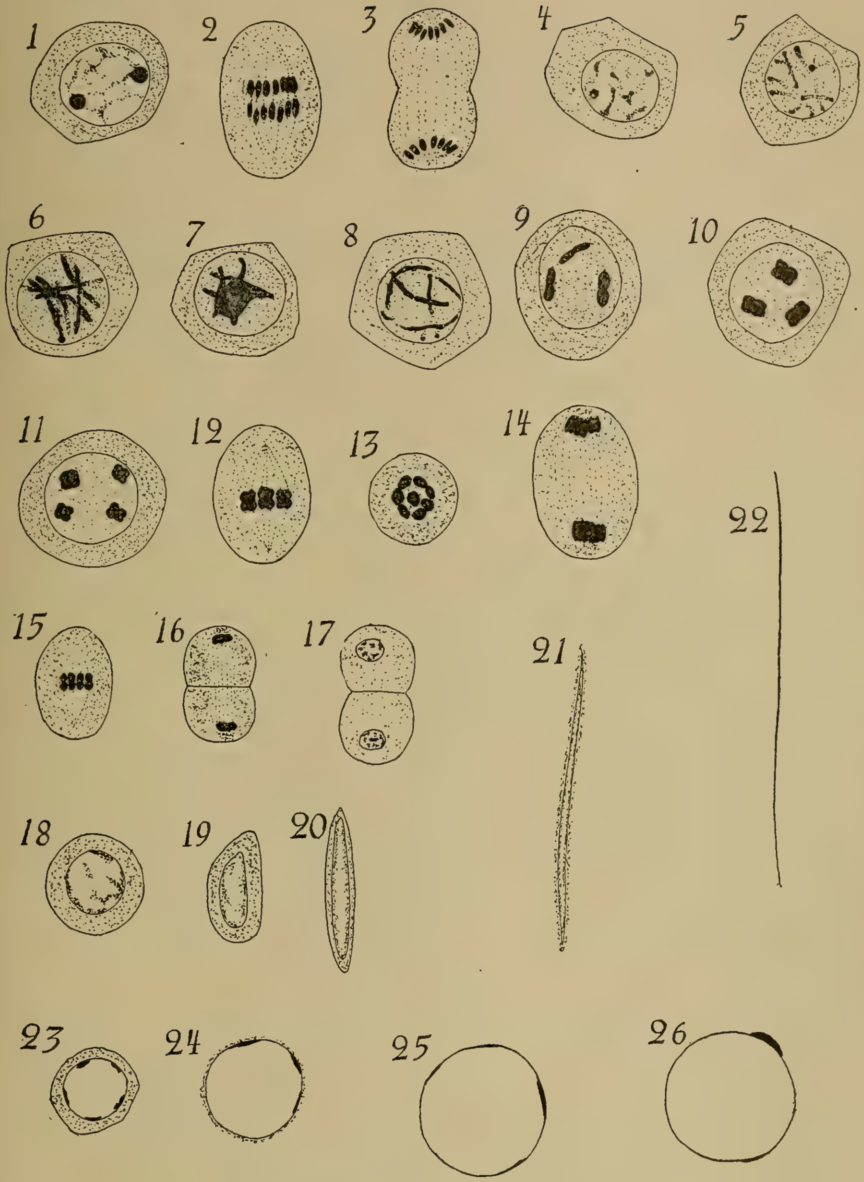
In Fig. 23 the cytoplasm has decreased in amount and the chromatin has collected into lumps at the periphery of the nucleus.

In Fig. 24 the cytoplasm has disappeared save for a thin granular layer, and the nucleus is distended by the nutritive sphere.

In Fig. 25 the nutritive sphere has increased in size and become denser, while the cytoplasm has entirely disappeared.

In Fig. 26 the nuclear wall has disappeared and the chromatin forms lumps on the surface of the nutritive sphere.

PLATE I


1
2
3
4
5
6
7
8
9
10
11
12
13
14
22
15
16
17
21
18
19
20
23
24
25
26

THE ORDER OF APPEARANCE OF THE ANTERIOR SOMITES IN THE CHICK.[1]

J. THOS. PATTERSON.

A. INTRODUCTION.

The statement that in the chick, somites arise in front of the first somite formed in the series has been widely accepted by embryologists. This view, nevertheless, is not in accord with our knowledge concerning the early development of birds, for it is well known that differentiation usually begins at the anterior end and progresses posteriorly.

Although workers in this field agree that somites arise anterior to the one first formed, yet they differ as to the exact number. Thus Balfour ('85) states that there is one, while His ('68) and von Baer ('28) have estimated it at two. Kupffer and Benecke ('79) would lead one to believe that there were at least three or four. So far as I am aware the latest work done to determine this number is by Miss Platt ('89), who concludes from a study of sections that there are two, or, to be more exact, one and a half.

From the results of certain experiments, made in connection with an experimental study of the early development of the pigeon, the writer was led to believe that *no* somites were formed in front of the first mesodermic cleft, except, of course, the so-called rudimentary or incomplete anterior cephalic somite. At the suggestion of Professor Lillie I have performed a number of experiments to test the validity of this view. These experiments, in connection with others, were conducted on a farm in Ohio, where I had at my disposal the eggs from fifty laying hens. It was possible, therefore, to collect and incubate the eggs hourly.

It gives me pleasure here to express my thanks to Professor Lillie for his kindness in sending all the necessary equipment for this work from these laboratories, and for his valuable criticisms.

---

[1] Unless otherwise indicated, the word somite will be used throughout this work to mean protovertebra.

B. METHODS.

For opening and sealing the egg I have in the main employed the method first used by Miss Peebles ('98). By the aid of a fine file a small window is made in the shell just above the blastoderm. The operation is then performed and the opening closed with a slightly larger piece of shell (with membrane still attached) from the corresponding part of a fresh egg.

Although I have used very fine glass pins in some of the work, yet I have found the electric needle by far the better means for making the injury. These needles (No. 12 sewing needles, ground as fine as possible on a water stone) were connected with two dry battery cells.[1] Then, by the aid of a binocular, using a combination of lenses giving a magnification of 12.6 diameters, one needle is placed at the desired point and the other is touched to the albumen for a second or two. The opening is then closed in the manner stated above. The whole procedure from the opening to the closing of the egg need not take over a minute.

If no further precautions were necessary the experimental work would be a simple process, but the difficulties that attend experimental studies on the bird's egg are many. Perhaps there is none so perplexing as that of preventing infection. Previous workers have realized this fact. Some writers have reported a loss of embryos, due to mould or bacteria, reaching as high as 80 per cent.

The mere heating of the instruments is not sufficient in itself to prevent infection. However, I find that if one uses a .1 per cent. solution of bichloride of mercury previous to heating, this difficulty is practically overcome. The table upon which the operation is performed, the hands, the instruments, in fact, every thing connected with the operation must be thoroughly washed in this solution. With a cloth moistened in the sublimate I also wipe off the shell where the window is to be made, otherwise small fragments of the shell falling upon the albumen will be a frequent source of infection.

---

[1] The Cleveland Dry Battery Cells were used. Each cell has a pressure of about 1.3 volts.

[2] All the drawings with which this paper is illustrated were made by the aid of the Abbe camera.

After closing the opening I place over it a piece of sterilized cotton about 5 cm. square and holding down the edges of the cotton on the sides of the shell, slowly revolve the egg until the closed window is on the lower side. It is then placed in a watch-glass and incubated the desired period of time. In addition to holding the piece of shell in place, the cotton prevents the egg from rolling and facilitates handling.

Inverting the egg serves a double purpose. In the first place, no matter how careful one may be a certain number of eggs are sure to become infected by germs falling on the albumen from the air. Now, when the egg is revolved, the yolk turns until the blastoderm is uppermost and hence removed as far as possible from the region of possible infection, which spreads too slowly to reach the blastoderm and interfere with the development of the embryo, especially if the egg is incubated but a few hours. In the second place, by revolving the egg the blastoderm is brought into an environment almost, if not entirely, normal. Mitrophanow ('97) has shown that varnishing the shell above the blastoderm retards development by limiting the supply of oxygen, and produces abnormalities. A similar effect is undoubtedly produced by placing an extra piece of shell above the embryo and sealing down its edges with strips of membrane — a method used by some workers. Any abnormalities thus produced are to be avoided, because they complicate the correct interpretation of one's experimenal results.

In order to test whether inverting the egg brings the blastoderm into a normal environment, some eggs were thus turned, while others were allowed to remain with the covered window uppermost, or turned but slightly to one side. In general, the latter were delayed from two to four hours, while the former developed equally with the controls.

It may seem that the above precautions are a bit tedious and unnecessary, but one is fully repaid for the trouble thus taken, as shown by the following statistics. During the period in which this series of experiments was carried on over 400 operations were made and but five eggs were infected, or less than 2 per cent., and during the present year about 100 operations have been performed with not a single case of infection noted. Of this entire number between 80 and 90 per cent. of the eggs have given definite results.

C. Experiments.

If only the rudimentary somite arises in front of the first mesodermic cleft, an injury made just anterior to this cleft ought to destroy, at least partially, this incomplete somite; but if, in addition, complete somites are formed anterior to this cleft, the injury ought to appear later in that one of the complete somites lying just in front of the cleft.

In performing such an operation there are two sources of difficulty. In the first place, owing to the individual variation in the early development of eggs, it is very difficult to hit upon the exact time when but one cleft is present. This difficulty can be met by opening the egg one to two hours before the cleft ordinarily appears and temporarily sealing the opening, so that it may be reopened and examined from time to time until the cleft appears. It was found that the average time of appearance of this cleft is between 21 and 22 hours. In the second place, it is almost impossible in a large number of embryos, to see the cleft so that one may be sure of the operation. This is due to the fact that the yolk is often a very pale yellow, and the white embryo cannot be seen. However, in about 20 per cent. of the eggs the yolk is a deep yellow, and against this background the embryo stands out in perfect contrast, and one can be absolutely certain of one's operation. In this work only the latter kind of eggs was used.

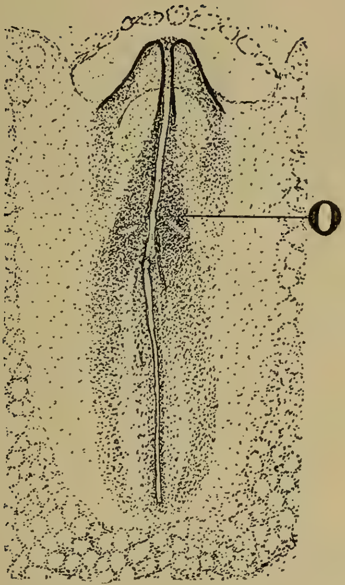
Experiment I.

The operation was performed with an electric needle after the egg had been incubated 22 hours at a temperature of 37–39° C. Fig. 1 illustrates the place of injury (Fig. 1, *O*) and the condition of the embryo at the time of the operation. It will be noted that the first cleft lies just anterior to the fore-end of the primitive streak and meets the main axis of the embryo at an oblique angle.

After the operation the egg was incubated ten hours. The embryo was killed in picrosulphuric-acetic acid, stained in Conklin's picro-hæmatoxylin, and mounted in xylol-balsam.

Fig. 2 shows the result of the operation. On the right side the rudimentary somite is greatly disturbed (Fig. 2, *O*) and the neural tube is slightly injured. Aside from this the embryo is normal in every way. The eighth pair of somites is just being cut off and the heart is forming.

A sagittal section through the somites of this embryo confirms what is seen in surface view. The rudimentary somite is greatly injured (Fig. 3, *x*) and its characteristic enlargement (see Figs. 10–12) is not present.



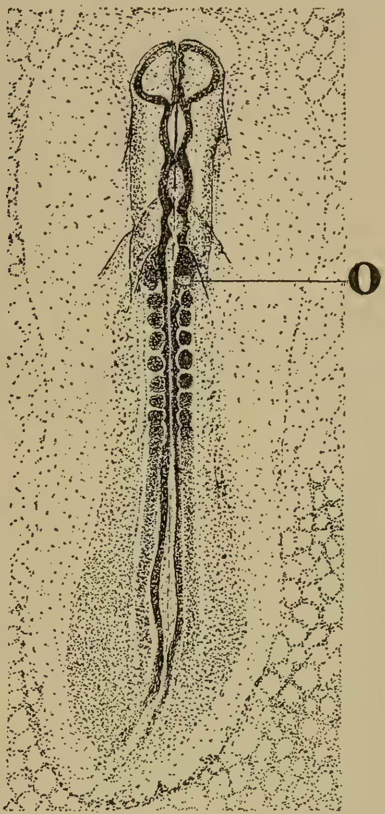


FIG. 1. Twenty-one hours old. It shows the first pair of clefts, which meet the main axis of the embryo at an oblique angle. The place of injury is shown at *O*. × 20.

FIG. 2. Twenty-two hours old when operated on, and then incubated ten hours. It shows eight pairs of somites, and the injury in the right anterior somite at *O*. × 20.

EXPERIMENT II.

The conditions under which the operation was performed and the subsequent handling of the embryo were the same as in the preceding experiment. Instead of using the electric needle, a very fine glass "pin" was substituted. This pin was placed just in front of the first cleft on the left side at a stage corresponding to that of Fig. 1. After the operation the egg was incubated twenty and one half hours longer.

The result of the operation is shown in Fig. 4. The needle is found in the incomplete somite on the left side (Fig. 4, *O*). There are 16 pairs of somites and the heart is well formed. It was impossible to section this embryo on account of the glass pin, but the surface view is so clear that there can be no doubt as to the position of the pin.

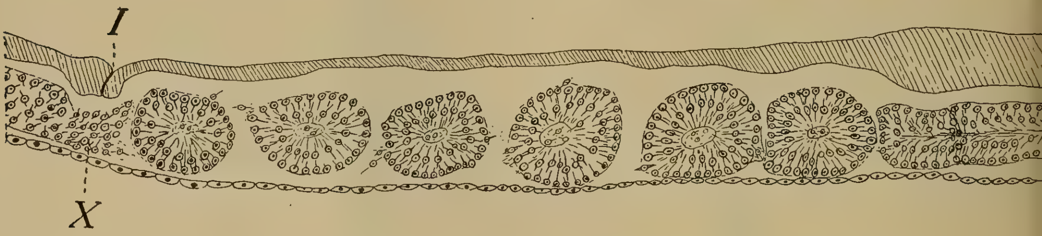


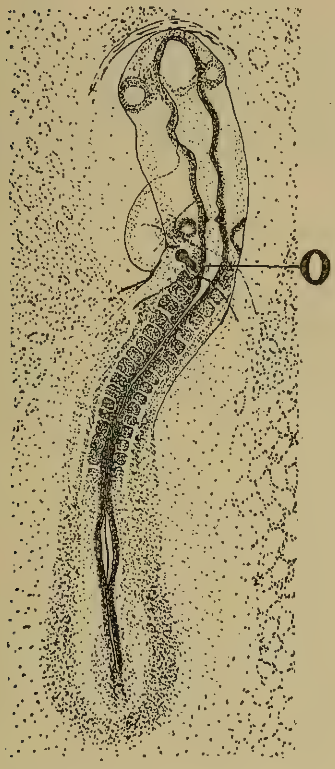
FIG. 3. Sagittal section through the somites on the right side of the embryo represented in Fig. 2. It shows at *X* the destroyed incomplete somite and at *I* the place where the needle has broken through the ectoderm. × 157.

EXPERIMENT III.

In order to show that the mesoderm lying between the injury and the cleft (between *r* and *a*, Fig. 6) does not increase subsequent to the operation and antecedent to the examination of the result and give rise to one or more somites, the injury was made so as to destroy the mesoderm spanning the first cleft (Fig. 6, *a*).

The result of such an experiment is well illustrated in Fig. 5. This embryo was treated in exactly the same manner as the one represented in Fig. 2. In addition to destroying the posterior edge of the incomplete somite, the injury extends over a portion of the cleft (Fig. 5, *O*). In this, as in Fig. 2, sections confirm what is seen in surface view.

The above are only a few types out of about seventy-five experiments performed to throw light on the order of development of the somites. In all cases the results support the view that only the incomplete somite arises anterior to the first mesodermic cleft. In the cases cited, the oldest embryo was carried to the sixteen-somite stage, but other embryos were allowed to develop until twenty or twenty-five somites appeared, with the injury still found in the rudimentary somite. In another series the injury was made just posterior to the first cleft. In such cases the first complete somite was either greatly disturbed or entirely destroyed.



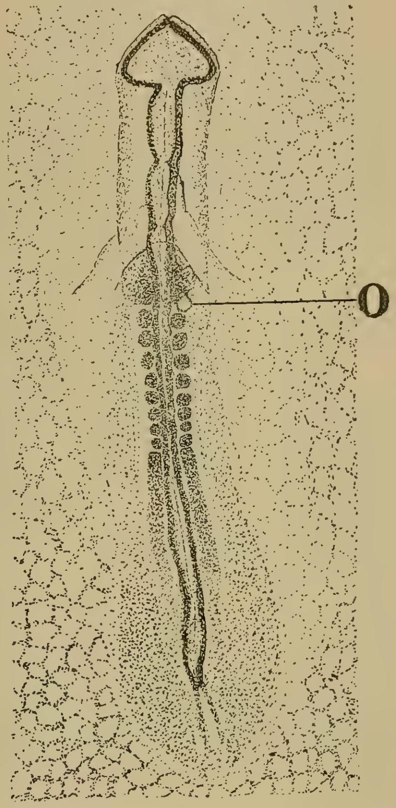


FIG. 4. Twenty-two hours old when operated on, and then incubated twenty and one half hours. There are sixteen pairs of somites. The glass pin is located in the incomplete somite on the left side, at *O*. × 20.

FIG. 5. Twenty-two hours old when operated on, and then incubated ten hours. The injury is shown at *O*, and ten pairs of somites are present. × 20.

D. STUDY OF SAGITTAL SECTIONS.

The results of the above series of experiments are in themselves sufficient to prove the position taken in this paper. But inasmuch as some writers have drawn their conclusions from a study of sections, it seems advisable to introduce a few figures to show that sections support the above contention. By far the most critical study made on sections is by Miss Platt, whose view is well summed up in her conclusion, in which she says : "My conclusions are, therefore, that the first break in the mesoderm occurs anterior to the first protovertebra, and that two protovertebræ (or, more correctly, one and a half) are slowly formed

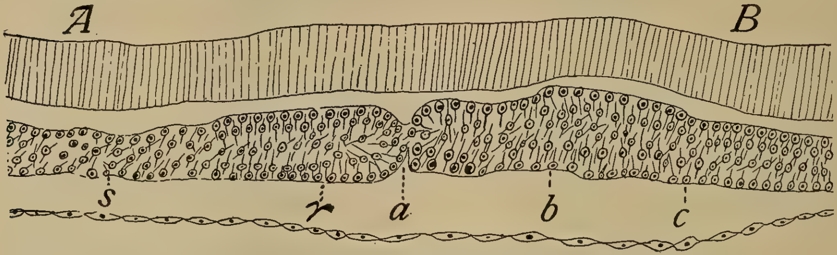


FIG. 6. Shows the first mesodermic cleft, and there are also indications of the second and third clefts. *r*, beginning of the rudimentary somite. × 247.

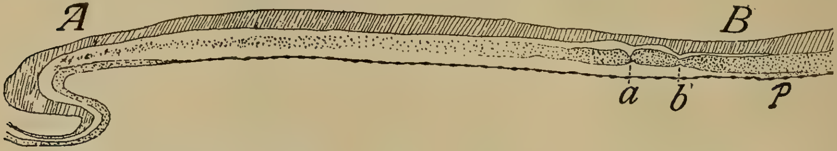


FIG. 7. Introduced to show the relation of the first and second clefts to the rest of the embryo. *p*, anterior end of the primitive streak; *b–p*, region of differentiation of the embryo. × 68.

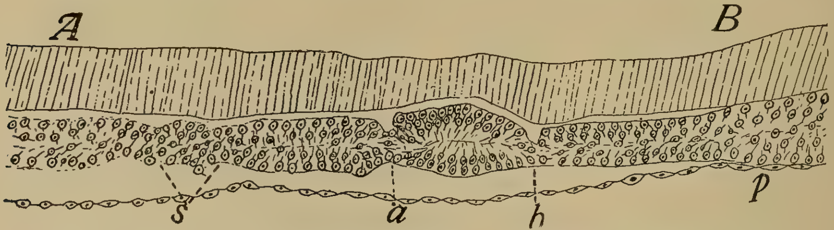


FIG. 8. Enlarged portion of the opposite side of the embryo represented in Fig. 7. *s*, shallow depression. × 247.

FIGS. 6–8. In these, as in the remaining figures of this paper, *A* is anterior and *B* posterior end, and *a*, *b*, *c*, etc., are respectively the first, second, third, etc., clefts.

nterior to the first mesodermic cleft, in the time occupied by the formation of six or seven protovertebræ posterior to that cleft."[1]

The first indication of somites is a depression on the dorsal surface of the mesoderm lying at the sides of the notochord, just anterior to the fore-end of the primitive streak. This is soon followed by a corresponding indentation on the under side (Figs. 6 and 7, *a*). Between the cleft and the anterior end of the primitive streak, indications of the two succeeding clefts are already present (Fig. 6, *b* and *c*). In front of the cleft, the strip of mesoderm which extends forward into the head gradually thins out anteriorly. At some points this thinning out has progressed more rapidly than at others, giving rise to shallow, transitory depressions (Figs. 6 and 8, *s*), which have been wrongly interpreted as clefts by Miss Platt.

The mesoderm immediately anterior to the first cleft is destined to form the rudimentary somite, and the manner in which this structure arises can be followed with no small degree of certainty. At first the mesoderm is quite uniformly thick (Fig. 9, *t*), but by the time three or four somites are formed its posterior edge has become much enlarged. Apparently this thickening takes place at the expense of the mesoderm just anterior to it (cf. Figs. 9–11, *t*). It should be remembered, however, that this incomplete somite is never so large as the others, really being, as Miss Platt states, only a half-somite. It must be considered as a part of the head mesoderm, from which it never becomes separated (Figs. 9–12, *r*).

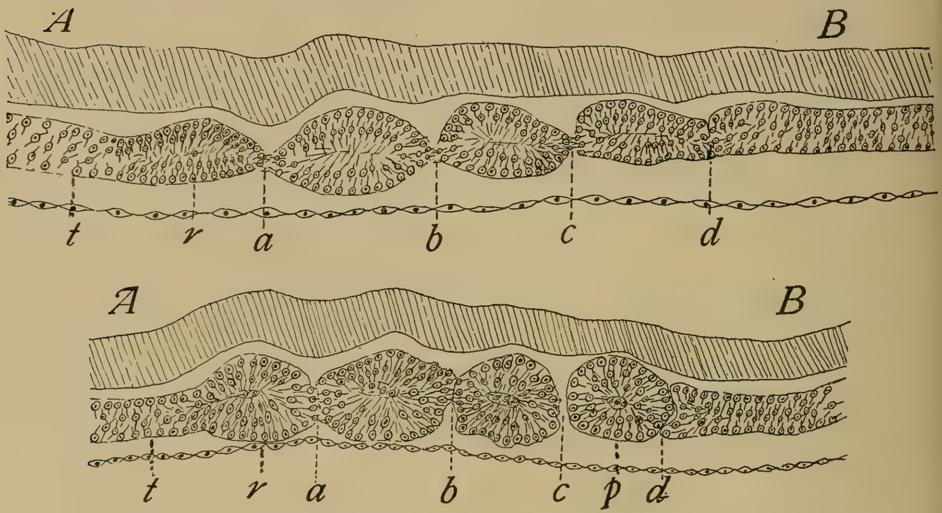
Miss Platt used the relative depths of the clefts as a means for determining the priority of somites. After very justly criticising Kupffer for judging either the fourth or fifth somite to be the oldest on account of its size, she says: "I think enough has been said to show that neither the size of the protovertebræ, their relative distance from the primitive streak, nor yet their obliquity to the main axis, is a sufficient ground to warrant a decisive answer to the question in regard to the order of their development,"[2] and I should add, that neither can the depth of the cleft be taken as a criterion for ascertaining seniority. The error into which one

[1] *Loc cit.*, p. 178.

[2] *Loc. cit.*, p. 174

falls by using such an index, becomes apparent on examination of sections such as are represented in Figs. 9 and 10. In Fig. 9, clefts *a*, *b*, and *c* are so nearly alike that it would be impossible to say which is the oldest, judging from their depths.

In Fig. 10 cleft *b* is slightly deeper than *a*, but *c* is still deeper. In such cases, those who accept the relative depths of the clefts as a criterion for drawing conclusions, would be forced to say that two and one half somites arise anterior to the first formed somite, because, as Miss Platt correctly states, "the first cleft lies anterior to the first protovertebra, not posterior, as Kupffer and Benecke supposed."[1] In other cases I have observed the fourth cleft to be the deepest.



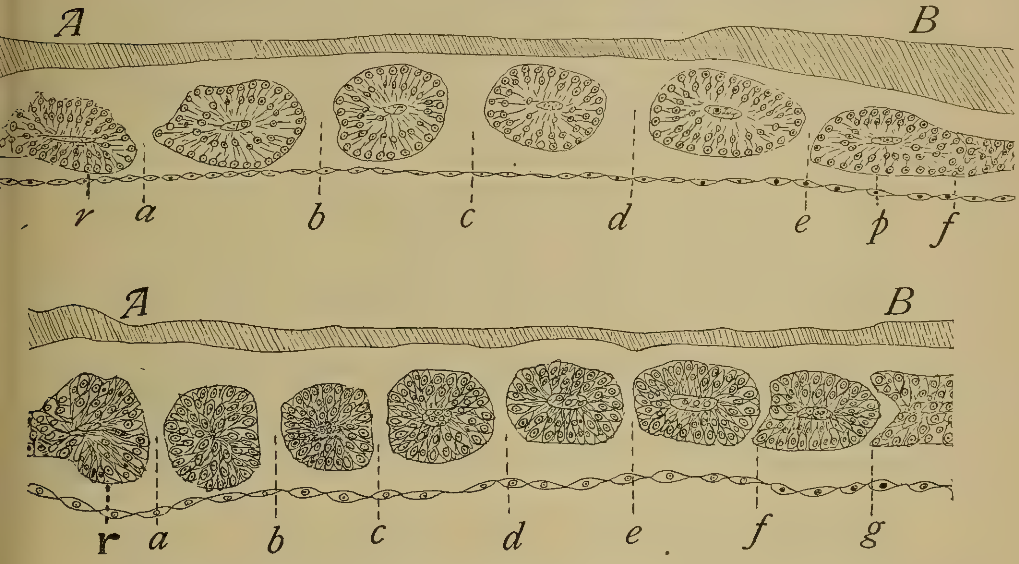
FIGS. 9 and 10. Sections of three and four somites respectively. Both figures show the failure of the first clefts to cut off completely the anterior somites in the beginning. × 157.

The condition seen in Fig. 10 is a very common one, and is brought about by the manner in which somites posterior to the first two or three are cut off. In the beginning the first clefts never completely separate their bordering somites, so that the anterior somites often remain connected until the sixth or seventh pair is formed, but posterior to these anterior clefts, succeeding somites are delimited, often before there are any indentations on

---

[1] *Loc. cit.*, p. 176.

the upper or under surface of the mesoderm (Fig. 11, *f*). As seen in section, the forming somite is at first elongated, with its long axis coinciding with that of the embryo (Fig. 11, *p*). However, it soon rounds up (Fig. 10, *p*), and in so doing becomes separated from the posterior mesoderm (Fig. 12, *g*). The cleft thus made is never vertical, but is so formed that the posterior edge of the last formed somite is in the shape of a wedge, which fits into a corresponding concavity on the anterior edge of the unsegmented mesoderm (Figs. 10–12). That the above process of cutting off somites is a rapid one, is shown in Fig. 10, in which it will be noted that the somite is completely formed before there is any indication of one succeeding it.



FIGS. 11 and 12. Sections of six and seven somites respectively. *r*, rudimentary somite. × 157.

E. Discussion and Conclusion.

Since Miss Platt's account of the formation of the incomplete somite is not essentially different from that of mine, it follows that we differ only as regards one cleft. According to Miss Platt's view one cleft slowly forms in front of the first one, and hence one complete somite arises anterior to the first formed somite.

I have shown that this author has mistaken the most posterior of certain transitory shallow depressions in the head mesoderm for a cleft. I have also made it clear that the first few (2 or 3) clefts are not completed until six or seven pairs of somites are formed. It seems reasonable to suppose, therefore, that Miss Platt has interpreted the first cleft, during the early stages of development, as a derivative of the most posterior shallow depression. It should be added that this posterior shallow depression persists longer than the others.

Since these shallow transitory depressions are situated at regular intervals, I might suggest that they lend themselves to another interpretation, namely, as vestigial clefts separating the cephalic mesoblastic somites. Notwithstanding the fact that Locy ('95) and his followers minimize the value of myotomes in ascertaining the metamerism of the vertebrate head and use neuromeres as the *sine qua non* for determining primitive segmentation, nevertheless the glimpses one gets of such structures as that cited above should not be overlooked. In fact, if these vestigial clefts are studied in connection with the various conditions seen in the myotomes, the above interpretation becomes evident, for in passing backwards from the anterior end of the embryo one finds that the clefts become more and more pronounced. This is evidenced by (1) the vestigial clefts, (2) the rudimentary somite, whose anterior cleft fails to separate it from the head mesoderm, (3) the slowness of the first clefts in cutting off the anterior protovertebræ, (4) and finally the sharpness and rapidity with which all succeeding protovertebræ are cut off. In other words the influence of the process which has completely obliterated or greatly modified the anterior cephalic somites, gradually becomes weaker in passing posteriorly, and finally ceases altogether.

In regard to the experimental work it seems unnecessary to add to what has already been said. It was noted that an injury made, either with a glass pin or an electric needle, just anterior to the first cleft, appeared upon further incubation, in the rudimentary somite, showing beyond a shadow of doubt that no somite, except the incomplete one, is formed anterior to the first mesodermic cleft. This brings the order of the appearance of somites of the chick into harmony with the general law for the early development of the embryo, namely, that differentiation begins at the anterior end and progresses posteriorly.

Hull Zoölogical Laboratory,
University of Chicago,
April 3, 1907.

LITERATURE CITED.

Balfour, F. M.
'85 Comparative Embryology.

His, W.
'68 Untersuchungen über die Erste Anlage des Wirbelthierleibes. Leipzig.

Kupffer, C., and Benecke, B.
'79 Photogramme zur Ontogenie der Vögel. Halle.

Mitrophanow, P.
'97 Teratogenetische Studiens, II. Archiv f. Entwickelungsmechanik d. Organisem, Bd. VI., Heft 1.

Peebles, Florence.
'98 Some Experiments on the Primitive Streak of the Chick. Archiv f. Entw.-mech., Bd. VII., s. 405–429.

Platt, Julia B.
'89 Studies on the Primitive Axial Segmentation of the Chick. Bull. of the Mus. of Comp. Zoöl. Harvard Coll. Vol. XVII., No. 4.

Von Baer.
'28 Entwickelungsgeschichte der Thier.

SUGGESTED EXPLANATIONS OF CERTAIN PHENOMENA IN THE LIVES OF ANTS; WITH A METHOD OF TRACING ANTS TO THEIR RESPECTIVE COMMUNITIES.

ADELE M. FIELDE.

A. During summers spent upon Cape Cod and Cape Ann, I have observed a preponderance of queen-pupæ in the nests of myrmicine and camponotine ants in the month of June, and a marked diminution or absence of such pupæ late in summer. This observation has led me to believe that queens may be the issue of eggs deposited in the preceding summer, having passed the winter in the larval stage in company with the hibernating ant-nurses. They would thus receive the earliest attention of these nurses at the beginning of the warm season and would then acquire the size and traits that distinguish them from the workers, the workers being more rapidly developed from eggs deposited during the summer in which they hatch. It is well established that the activities of the ants increase with the temperature[1] up to 85° F. or 30° C. The maximum activity of the nurses, with the more abundant food-supply in summer, should make queen-pupæ more numerous in early autumn than in early summer, were these pupæ the product of eggs deposited during the summer in which the queen-pupæ are discovered. The relative paucity of insect food, the comparative inactivity of the ant-nurses during the spring, and the brevity of the interval between the emergence of the ants from the hibernating place and the discovery of the queen-pupæ, render it fairly certain that these pupæ had attained the larval stage previous to the retirement of the ants into the deep recesses of their nest at the approach of the preceding winter.

---

[1] "Observations on Ants in their Relation to Temperature and Submergence," A. M. Fielde; BIOLOGICAL BULLETIN, Vol. VII., No. 3, August, 1904, and "Temperature as a Factor in the Development of Ants," A. M. Fielde, BIOLOGICAL BULLETIN, Vol. IX., No. 6, November, 1905.

B. Last summer, on Cape Ann, I kept a colony of formicid ants, that were living in their natural nest under a single stone, abundantly supplied with crushed insects, sugar, and sponge-cake, the supply being protected from rain and renewed at least twice a week. I also arranged annexes to the nest, and these proved so acceptable to the ants that they moved part of their colony into my additions to their residence. At the end of June, when I first observed this nest, there was a great number of queen-pupæ in it but no queen was visible to me. Early in July there were numerous newly hatched queens, and from that time until the eighth of September, when I left Cape Ann, the queens were countless, and there had been no observed swarming from the nest. Somewhat similar observations made by me upon other natural nests have suggested the possibility that the retention of more than one queen by certain species of ants, including *Stenamma fulvum* may result from abundance of nutriment, attainable without excessive labor on the part of the workers.

C. In my formicaries, ants of two species, *Lasius latipes* and *Camponotus herculaneus*, neither of which is a tent-builder, as well as the tent-building ant, *Cremastogaster lineolata*, have at different times protected their young from light by making, during the night, a continuous layer of small pellets of earth on the top of a pane of transparent glass, that I had placed horizontally over a hollow in which the young were assembled. Since all ants habitually withdraw their young from the ultra-violet rays of light, it appears probable that the tent-building ants erect their peculiar structures for the purpose of shielding their young from these rays; and the above recorded observations, on ants of another subfamily, indicate that specific conditions may impel other than tent-building ants to become tent-builders.

D. For correct interpretation of the behavior of ants observation needs be indefinitely prolonged. Sometimes the real animus of one ant toward another is revealed only after weeks or months of continuous association. Some ant-sisters, *Camponotus pennsylvanicus*, reared by me and indisputably the offspring of the same queen-mother, were separated all their lives, the younger in one group, and their elders by a year in another group. The two groups were gradually and cautiously made acquainted with one another, and the younger sisters were supposed by me to have become reconciled to the progressive odor of their seniors. But after being united in an apparently congenial family group, a senior worker was occasionally killed by a junior, and successive conflicts utterly destroyed the colony after several months.

I have repeatedly observed the gradual dwindling and extinction of apparently healthy ant-groups in which the individuals bore odors not wholly familiar to all the inhabitants. Into an artificial nest of *Camponotus herculaneus*, in which the occupants were all virgin workers, who had never before met a male of their species, I introduced several males. The first impression of an inexperienced observer would probably have been that the workers attacked the males with intent to tear them limb from limb. For some hours the attacks were maintained; but the males remained unscathed and, without even a rent in their delicate wings, continued for weeks in close companionship with the workers. It was attraction, not antipathy, that dictated the violent behavior of the workers.

The presence of young; the completeness of the establishment of the nest-aura; the domestic conditions in general; the familiarity of the ants with their immediate environment; the incurred odor; the inherited odor; the progressive odor; the specific odor; the sensitivity of the ants to a preponderating odor and their encouragement or discouragement therefrom; the aptness of ants to concentrate attention upon an immediate interest and to become temporarily oblivious to other matters; and the associative memory maintained by every ant concerning its previous experiences, are all factors which need be weighed when determining the causes of the behavior of ants.

E. When making inquiry of the ants concerning their sense of hearing in the summer of 1903, I deprived the ants of portions of their bodies and found that the excision of certain parts uniformly affected the direction of the movement of the ants when they were startled.[1] Normal queens (of *Stenamma fulvum*) moved either forward, backward or sidewise, while queens deprived of both antennæ invariably moved backward or sidewise, never forward, and queens deprived of the abdomen always moved forward or sidewise. Since the publication of that paper, it has appeared to me probable that the uniform differences in the direction of movement coincident with uniform maiming of the ants, might be explained by the change of the location of the center of gravity within the body of the ant. Change of the location of the center of gravity in the body of a queen upon the loss of her wings after mating may also explain certain changes observable at that period in her characteristic behavior. The retiring tendency of the queen after deälation may be due to the change in her center of gravity.

---

[1] "The Reactions of Ants to Material Vibrations," by Adele M. Fielde and George H. Parker, *Proceedings of the Academy of Natural Sciences of Philadelphia*, November, 1904, p. 646.

F. Dr. H. A. Parr, of New York, has mentioned to me an unpublished method used by him in tracking ants to their respective colonies. From a fleck of raw cotton he makes a minute torch-shaped ensign, colors the bluffy end in an anilin dye, and dips the hard-twisted handle into melted sugar. Ants will pick up this flag-like object, hold it by its sweet handle, and carry it homeward. Being very light and flexible, it does not greatly hinder the bearer in her progress through grasses and among stones; the brilliant pennon is easily followed by the eye of the observer; and different ants are distinguished by the different colors that they carry. This device enables the observer to track ants through long distances and to ascertain whether those discovered at a common rendezvous belong to one or to diverse communities.

New York City,
April, 1907.

STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS.

III. Maturation, Fertilizaton, and Cleavage in Moniezia.

C. M. CHILD.

Since the present paper is concerned primarily with the rôle played by amitosis and mitosis, respectively in the later history of the ovum and the early development of *Moniezia*, the stages of egg-maturation and fertilization are in themselves of secondary importance. It has seemed advisable, however, to include an account of these stages, although it has not been possible to attain certainty on a number of points, *e. g.*, the polarity of the egg before maturation, the direction of division of the chromosomes in maturation, the origin of the cleavage centrosomes, etc. Failure to reach definite conclusions upon these points is due, at least in large measure, as will appear, to the character of the material rather than to insufficient observation. Much time and labor has been expended in the attempt to obtain positive data upon these points but thus far without success.

The cleavage of the egg is considered from a cytological rather than a morphological standpoint, and much that is of importance embryologically is not discussed, as aside from the present purpose. All the figures of maturation, fertilization, and almost all of those of cleavage-stages are from *Moniezia expansa* but no essential differences have been discovered in the two species. The magnification is the same as that used in preceding papers of the series.[1]

I. Maturation of the Egg.

In point of time the entrance of the spermatozoön precedes the process of maturation and the growth of the male pronucleus occurs during the formation of the polar bodies. For the sake of clearness, however, the two processes are described separately, the figures being sufficient to show the conditions as regards both maturation and fertilization at different stages.

---

[1] Child, C. M., "Studies on the Relation between Amitosis and Mitosis. I., Development of the Ovaries and Oögenesis in Moniezia." Biol. Bull., Vol. XII., No. 2, 1907. II., Development of the Testes and Spermatogenesis in Moniezia. Biol Bull., Vol. XII., Nos. 3 and 4, 1907.

When the egg leaves the ovary on its way to the uterus it is flattened or irregular in shape, without any visible polar differentiation so far as could be discovered, and contains a large nucleus with very large nucleolus. Within the nucleus all traces of the spireme which appeared at the beginning of the growth period[1] have disappeared and except for the nucleolus the nuclear contents, like those of many other egg-nuclei at this stage do not take nuclear stains.

Fig. 1 (Pl. II.) shows an egg at this stage but with the spermatozoön entering. In this egg two bodies shown below the nucleus are probably the centrosomes of the first maturation spindle, though it was impossible to be certain on this point.

With the entrance of the spermatozoön a vitelline membrane is formed (Fig. 2 et seq., Pl. II.). Chromosomes soon begin to form in the nucleus (Figs. 2 and 3, Pl. II.).

In Fig. 4 (Pl. II.) an early stage of the first maturation spindle is shown. Here the outline of the nucleus is still visible and the spindle appears to be wholly intranuclear. The very large centrosomes at the poles show a distinctly differentiated outer boundary which appears almost like a membrane. One of them in this figure shows two deeply staining granules, the other, none. The appearance and division of these central granules, or centrioles, is seemingly rather irregular as the following figures indicate. In no case has any trace of asters been observed at any stage of maturation, but with the formation of the spindle, the yolk spherules arrange themselves about the equatorial region. This arrangement of yolk spherules indicates that conditions about the poles of the spindles are similar to those in species where distinct asters appear. It is probable that the absence of asters is not due to any fundamental difference in the character of the processes in this case as compared with cases where asters are visible, but rather to the nature of the protoplasm or perhaps to the energy of the processes involved. The achromatic spindle-structures themselves are exceedingly delicate and often only

---

[2] Child, C. M., Biol. Bull, XII., 2, 1907.

very faintly visible. It is possible that some method of fixation which I have not employed may serve to render these phenomena more clearly visible. In any case, however, it seems improbable that the presence or absence of visible asters can be regarded as of essential importance.

It has not been possible to determine with certainty the number of chromosomes, though it is not far from eight, the most frequent number in the spermatocytes.[1] The arrangement of the chromosomes upon the spindle is very irregular and anything approaching a typical equatorial plate is rarely seen. The chromosomes exhibit the form characteristic of heterotypic divisions, but no conclusions were possible regarding the direction of division. Figs. 5 and 6 (Pl. II.) show other examples of the first maturation spindle. In Fig. 6 the extremely irregular passage of the chromatic material to the two poles is shown. The chromosomes often appear to be more or less broken up into chains of granules, which seem in some cases to become wholly separated from each other. It is impossible, of course, to determine by observation whether the chromosomes maintain their individuality during this process, but cases of this kind certainly do not appear to strengthen the hypothesis of individuality.

In some cases a nucleus with distinct membrane is formed after the first maturation division (Fig. 7, Pl. II.). In other cases, and apparently more frequently, the second polar spindle appears without an intervening resting stage (Fig. 8, Pl. II.).

The first polar body is of large size (Figs. 7 and 8, Pl. II.) and the centrosome is frequently visible beside the nucleus in it (Fig. 8, Pl. II., Figs. 9 and 10, Pl. III.). This centrosome stains more deeply than in earlier stages and still later apparently undergoes condensation to such an extent that it stains as deeply with iron-hæmatoxylin as a mass of chromatin or a yolk granule.

Fig. 8 (Pl. II.) represents an early stage of the second maturation spindle and Figs. 9 and 10 (Pl. III.) later stages. Fig. 10 is one of the very rare cases in which anything like a typical anaphase has been observed. The change in position of the spindle during its development is apparent by comparison of Fig. 8 (Pl. II.) with Fig. 9 (Pl. III.).

---

[1] Child, C. M., Biol. Bull., XII., 4, 1907.

The second polar body is, as is usual, smaller than the first (Fig. 11, Pl. III.) both as regards nucleus and cytoplasm. Division has not been observed in either polar body, though in one case what appeared to be an abnormal or abortive mitosis was seen in the first polar body.

Fig. 11 (Pl. III.) shows the reconstitution of the female pronucleus by the fusion of vesicles which doubtless arise from different chromosomes.

II. Fertilization.

The spermatheca opens into the oviduct at a point near the junction of the latter with the ovary. At the time when the eggs pass into the oviduct the spermatheca is distended with great numbers of spermatozoa a few of which are found in the narrow duct connecting spermatheca and oviduct. The spermatozoön meets the egg as it passes along the oviduct past the opening of the spermathecal duct.

Apparently the passage of the eggs through from the ovary to the uterus is periodical for among the large numbers of proglottids sectioned which contained cleavage-stages in the uterus and fully grown unfertilized eggs in the ovary only a very few show eggs in the oviduct. Among these, however, it has been possible to find a few cases showing the entrance of the sperm.

Fig. 1 (Pl. II.) shows the clearest case observed of this stage. It will be recalled[1] that the fully developed spermatozoön is greatly elongated and filiform without any trace of a visibly differentiated head-region, except that its diameter is slightly greater anteriorly than posteriorly.

In all cases where the spermatozoön was found on the egg it was in contact with the surface over more or less of its length, as if adhering to it. Frequently it was wound several times about the egg, the remaining portion hanging free in the oviduct or extending into the spermathecal duct.

In Fig. 1 the course of the spermatozoön body over the surface of the egg is indicated but only a fraction of the length of the spermatozoön is shown. The figure scarcely exaggerates the clearness of the section itself. That portion of the sperm which has entered the cytoplasm of the egg forms a deeply staining rounded mass in strong contrast to the remaining portions. Surrounding it is an area of cytoplasm staining less deeply than other portions of the egg — indicated in the figure by the dotted line. The appearance of the head-like structure is all the more remarkable since no trace of anything of the kind is visible before entrance. Evidently the anterior end of the spermatozoön loses its greatly elongated form after it enters the egg. It has been impossible to determine how much of the spermatozoön is involved in this change and also whether the remaining portions, if any, fuse with the cytoplasm or are cast off.

---

[1] Child, Biol. Bull., XII, 4, 1907.

It is impossible to distinguish with certainty the male pronucleus from small yoke-spherules after the "tail" of the spermatozoön disappears. The earliest stages observed with anything like certainty are shown in Figs. 2, 3 and 7 (Pl. II.). By the time the egg reaches the stage of the second polar spindle, however, the male pronucleus can usually be found in some part. In the eggs shown in Figs. 9 and 10 (Pl. III.) it was present in other sections.

By the time maturation is completed the male pronucleus has attained large size and shows a faintly staining reticulum with a nucleolus of large size. Figs. 11–14 (Pl. III.) show the two pronuclei at various stages of approximation to each other.

It has not been possible to obtain the slightest evidence in support of the view that the cleavage centrosomes arise from the spermatozoön. In the early stages of the male pronucleus (Figs. 2, 3 and 7, Pl. II.) no trace of spheres or centrosomes has been observed in connection with it. In a number of cases the two centrosomes have been observed lying near the pronuclei before cleavage (Figs. 12, 13, 14, Pl. III.) but in no case was there the slightest indication that they were more closely associated with one nucleus than with the other.

There can be little doubt from these observations that the peculiar spermatozoa of *Moniezia* actually fertilize the eggs and therefore that they contain nuclear substance in some form or condition, or at least substance capable of giving rise under proper conditions to a nucleus. If future investigation shall establish what seems at least possible from my own observations, viz., that functional spermatozoa arise from the "spermatid" nuclei, which themselves arise by fragmentation of the spermatocyte-nuclei,[1] we shall be forced to the conclusion that fertilization is possible without the typical process of maturation.

III. Cleavage.

The relations of the two pronuclei at the time of the first cleavage varies considerably in different eggs. In some cases the chromosomes form (Fig. 12, Pl. III.) and the membrane disappears while the pronuclei are still more or less widely separated. In such cases the chromosomes appear in two distinct groups on the two sides of the spindle (Figs. 15 and 16, Pl. IV.). In other cases the pronuclei approach more closely (Fig. 13, Pl. III.) or even undergo more or less complete fusion (Fig. 14, Pl. III.) before the disappearance of the membrane. In such cases the chromosomes may or may not appear in two groups according to the completeness of the fusion. In one case the chromosomes of the first cleavage were observed in two distinct groups within a single nuclear membrane (Fig. 17, Pl. IV.) and in many cases no trace of the paternal and maternal groups was visible (Figs. 18, 19, 20, Pl. IV.).

The position of the first cleavage-spindle varies greatly as is evident from Figs. 15, 16, 18, 19 and 20 (Pl. IV.). Since nuclear division proceeds much more rapidly than cytoplasmic division during early cleavage, and since the polar bodies soon degenerate or are absorbed, it has not been possible to determine whether the plane of the first cleavage is uniform in position, *i. e.*, whether the first cleavage-spindle finally attains a definite typical orientation in the egg. From my observations on cleavage stages I am somewhat inclined to doubt that this is the case, although my data are not conclusive.

There can be no doubt that the first cleavage is usually mitotic but occasionally conditions are found which might readily be regarded as cases of amitosis. Frequently eggs containing only a large single nucleus which is apparently giving rise amitotically to a smaller nucleus are found. None of these cases are figured since it is by no means certain that they are normal phenomena.

---

[1] Child, C. M., Biol. Bull., XII., 4, 1907.

In many proglottids a certain proportion of the cleaving eggs undergoes degeneration sooner or later, and such eggs usually show irregular nuclear fragmentations. It is therefore possible that the amitotic first cleavage may be an indication of degeneration. Possibly such eggs are not fertilized and are therefore incapable of normal development.

But although the first cleavage is usually or always mitotic, there can be no doubt that amitotic division appears very early in the course of cleavage.

In most cases a number of nuclear divisions occur before cell-boundaries become visible in the egg (Fig. 21, Pl. V.). Cases of mitosis are rarely seen after the first cleavage but amitosis is of frequent occurrence (Figs. 21–26, Pl. V.). It was impossible to determine whether the cleavage exhibited any regularity, for no basis for orientation was discovered. As cleavage proceeds, the egg is gradually divided into blastomeres containing yolk and blastomeres without yolk. In earlier stages the yolk-bearing blastomeres often contain two or more nuclei (Figs. 23, 26, Pl. V.), but in later stages after cytoplasmic cleavage is more advanced they usually contain one relatively large nucleus (Figs. 28, 29, 30, Pl. VI.). In other words as these yolk-bearing blastomeres are gradually reduced in size by successive cleavages the cytoplasmic cleavages keep pace more nearly with the nuclear divisions.

In the yolkless portions of the egg, however, nuclear division continues to be far in advance of cytoplasmic division as far as the cleavage has been followed (Figs. 27, 29, 30, 31, Pl. VI.; 32, Pl. VII.): the consequence is that each blastomere contains several or many nuclei of relatively small size. Evidently the nuclei in these yolkless portions of the egg are dividing much more rapidly than those in the yolk-bearing portions and, as is evident from the figures of Plates V. and VI., amitosis is the typical method of division.

Rarely a case of mitosis is observed: in all the hundreds of eggs in cleavage stages which have been examined, not more than a dozen cases of mitosis have been seen in stages later than the first cleavage. When mitosis occurs it apparently always involves one of the larger nuclei. Mitotic divisions of the small nuclei in stages like those shown in Plate VI. have never been observed. The smaller nuclei are, without doubt, dividing more rapidly than the larger, and we are probably justified in concluding that amitosis occurs in those regions of the egg where division is most rapid, while mitosis is found, when it occurs at all, among the nuclei which are dividing more slowly.

In Figs. 28 and 30 (Pl. VI.) two cases of mitosis are figured. In these two cases, in fact in every case of mitosis observed during later cleavage, the spindle lies within a blastomere which is bounded on all sides by a distinct membrane. This is particularly well shown in Fig. 30 where the blastomere undergoing mitosis forms a spherical mass with distinct membrane in the midst of the egg-syncytium. This fact, like others mentioned in preceding papers,[1] seems to indicate that conditions in regions where mitosis occurs are widely different from those in which nuclear division is amitotic. Evidently some physical or chemical condition is present in the region about the mitotic spindle in Fig. 30 which determines the formation of a cell-membrane about a certain mass of the cytoplasm. It is not impossible that the membrane may be a coagulation-product resulting from difference in electrical condition of the colloids in the two regions. Suggestions of this nature have been made by various authors, both as regards nuclear membranes and cell-membranes of this character.

As is evident from the figures, the embryos are quite irregular in shape. As cleavage proceeds, however, elongation commonly occurs, but whether this elongation is a mechanical deformation resulting from pressure of the uterine walls as the egg is forced through narrow openings, or whether it is a typical feature of morphogenesis is not certain. Such cases as Fig. 32 (Pl. VII.) in which one end of the embryo is drawn out into a sharp point seem to indicate mechanical deformation.

Even in these later stages of cleavage it has not been possible thus far to discover any certain basis for orientation of the embryo. In many cases one large yolk-bearing blastomere is found at each end of the elongated embryo (Fig. 27, Pl. VI.). In other cases only one such blastomere appears (Figs. 30, 31, Pl. VI.). My observations certainly indicate, though they are not sufficient to prove positively, that cleavage is indeterminate and exceedingly irregular.

---

[1] Child, Biol. Bull., XII., 2, 3, 4, 1907.

My material thus far has not included stages of development later than those shown in Plate VI. I hope in future, however, to obtain later stages and to continue the study of the rôle played by amitosis and mitosis in the later development.

In Figs. 33–40 (Pl. VII.) several single blastomeres and nuclei from various stages of cleavage are figured as showing particularly clear and convincing cases of amitosis.

IV. General Observations.

In this section a few observations of general biological interest though not connected with the chief purpose of the paper are briefly given. As was pointed out in the first paper of this series[1] the eggs in those portions of the ovary nearest the opening of the oviduct begin and complete their growth earlier than the others. Proceeding from this region toward the tips of the ovarian follicles, we find that oögenesis is slightly later at each successive level. Thus the eggs nearest the oviduct are the earliest, while those at the tips of the follicles are the latest to attain full growth.

There can be no doubt that the passage of ova from the ovary to the uterus is periodical and not of continual occurrence, for in most proglottids in which embryos are present in the uterus and immature eggs in the ovary, no eggs are found in the oviduct. In such proglottids nothing later than late maturation stages or cleavage stages is found in the uterus. Occasionally, however, a proglottid is found with eggs in the oviduct, and such cases show the entrance of the spermatozoa in those eggs in the oviduct and usually earlier maturation stages in the eggs in the lateral region of the uterus.

The egg usually reaches the lateral portions of the uterus before the appearance of the first maturation spindle. In the uterus, maturation stages and early cleavage stages become more or less mingled, although the maturation stages are usually more abundant in the lateral portions of the uterus and the cleavage stages in the middle portions.

[1] Child, C. M., Biol. Bull., XII., 2, 1907.

After passage of the eggs from ovary to uterus has occurred several times the different stages are mingled together in great confusion in the uterus for the uterus is now larger than in earlier stages and the eggs move more freely through it with the contractions of the body. In these proglottids particular stages are not confined to particular regions of the uterus: early maturation and later cleavage stages may occur side by side but even here the earlier stages are more abundant in the lateral and the later in the middle regions of the uterus.

That portion of the oviduct which lies between the entrance of the spermathecal duct and the uterus is highly convoluted and possesses thick walls, but no portion of it functions as a shell-gland. The egg passes into the uterus surrounded only by the vitelline membrane and this often disappears during cleavage.

Moreover, although a well developed vitellarium is present no yolk-cells pass into the uterus with the egg. To all appearances, the vitellarium undergoes degeneration *in situ*, though it is possible that yolk-cells may pass into the uterus in stages later than those which I have observed, or it may be that the reserve supplies of nutriment in the yolk-cells are altered by enzymes and so pass into the uterus in fluid form where they undergo resorption by the embryos.

It is very evident that the transference of the embryos of *Moniezia* to the intermediate host must occur either within the proglottid or in fluid since the absence of a shell or capsule of any kind would exclude the possibility of exposure to the atmosphere.

V. Conclusion.

Maturation and fertilization occur in *Moniezia* in a manner not essentially different from that observed in other forms. Evidently the earlier amitotic history of the germ-cells does not interfere in any way with their properties as germ-cells.

Moreover, although the embryonic development begins with mitosis, at least in most cases, if not in all, this soon gives place in large measure to amitosis, mitosis occurring only occasionally in the larger nuclei in the more slowly dividing regions. Here then, as in the development of the germ-cells, amitosis is apparently connected with more rapid, and mitosis with less rapid division.

The following paper will include a brief account of the rôle of amitosis in the organogeny of the proglottid, together with a general discussion and attempt at interpretation of the data presented regarding amitosis and mitosis in *Moniezia.*

April, 1907.

EXPLANATION OF PLATE II.

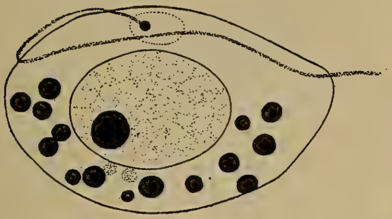
FIG. 1. The entrance of the spermatozoön.

FIGS. 2 and 3. Preparation for first maturation spindle; male pronucleus near surface of egg.

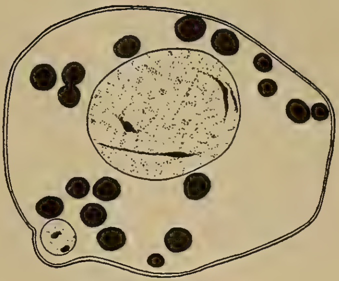
FIGS. 4, 5 and 6. First maturation spindle.

FIG. 7. Resting stage of oöcyte-nucleus before second maturation division.

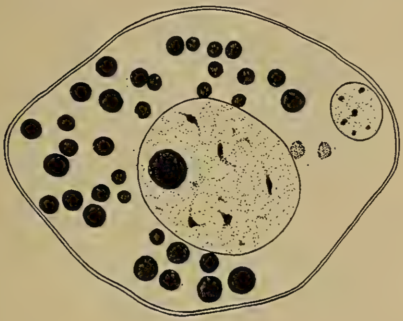
FIG. 8. Early stage of second maturation spindle.



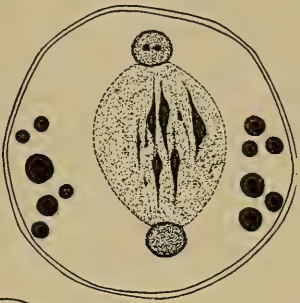
1



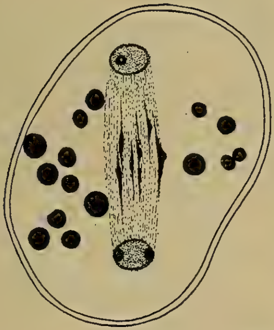
2



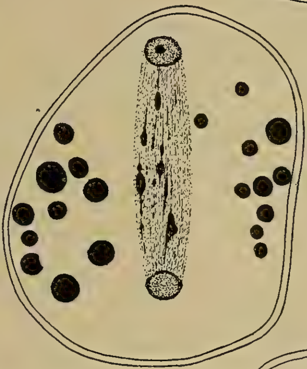
3



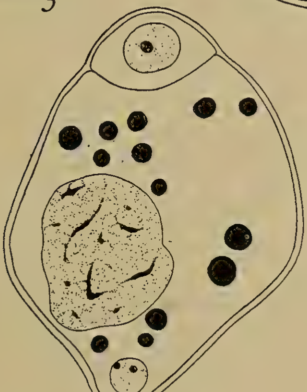
4



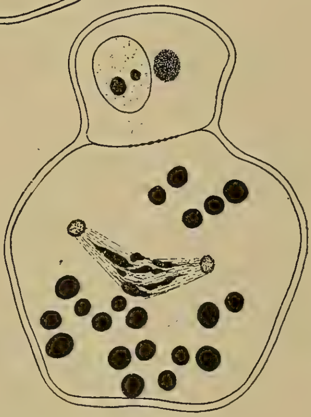
5



6



7



8

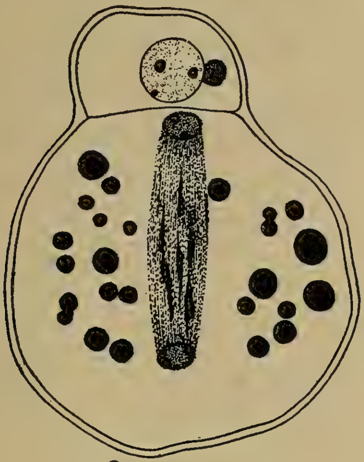
PLATE III.

FIG. 9. Second maturation spindle.

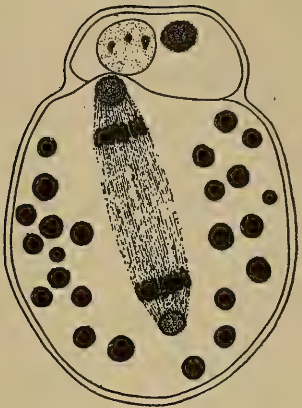
FIG. 10. Second maturation spindle; anaphase, a stage very rarely observed.

FIG. 11. Reconstitution of female pronucleus; male pronucleus on right.

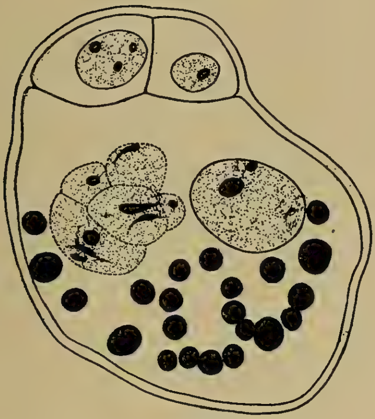
FIGS. 12, 13 and 14. Male and female pronucleus before first cleavage.



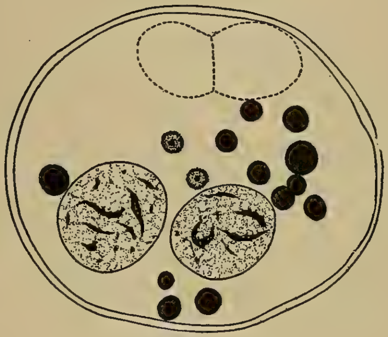
9



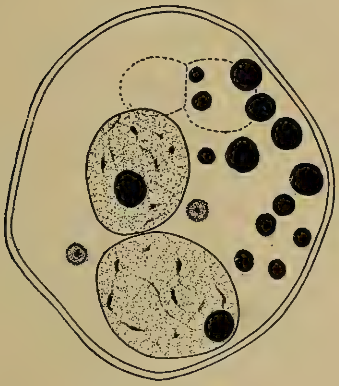
10



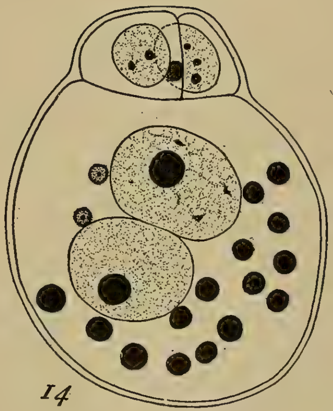
11



12



13



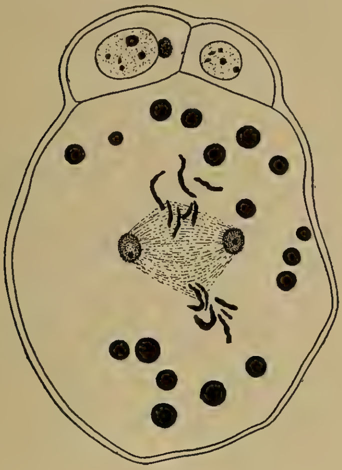
14

Plate IV.

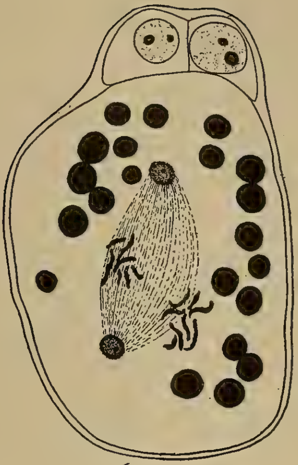
Figs. 15, 16 and 17. Early stages of first cleavage, showing distinct paternal and maternal groups of chromosomes, in Fig. 17 both within a single nucleus.

Figs. 18, 19 and 20. First cleavage, with chromosomes in a single group.

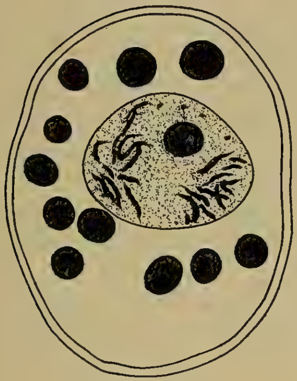
The figures show wide variation in the orientation of the first cleavage spindle.



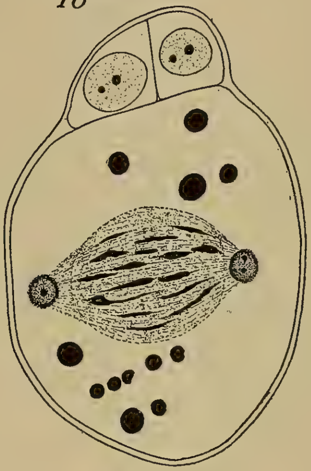
15



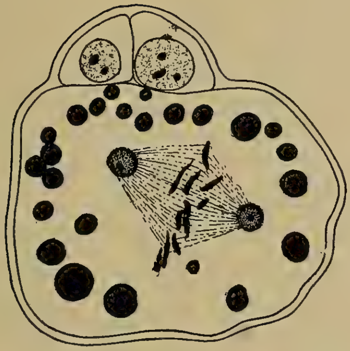
16



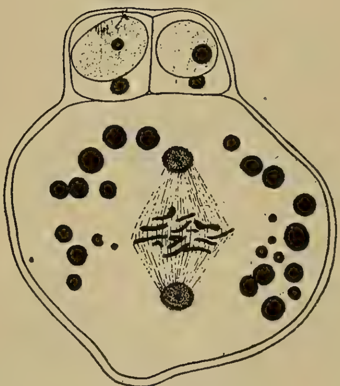
17



18



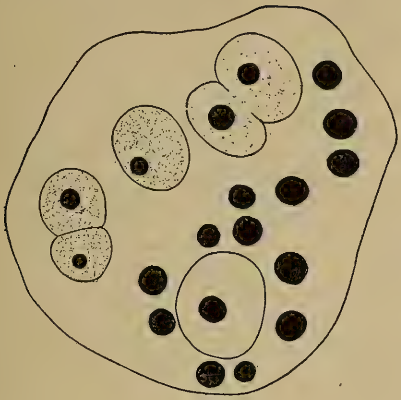
19



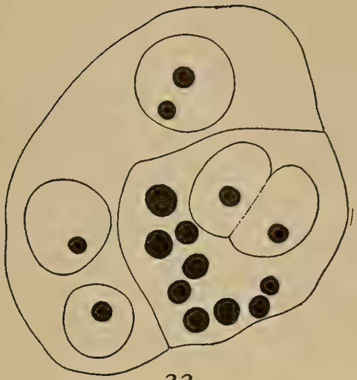
20

PLATE V.

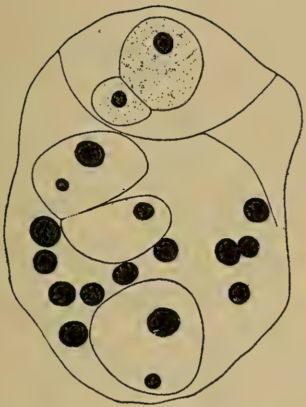
FIGS. 21–26. Early cleavage-stages.



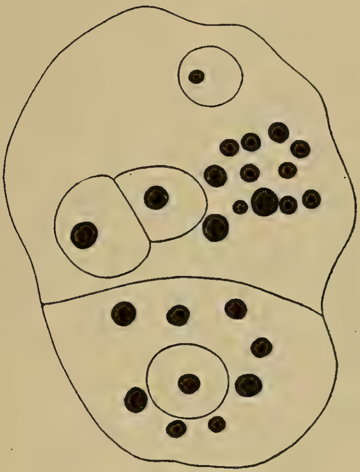
21



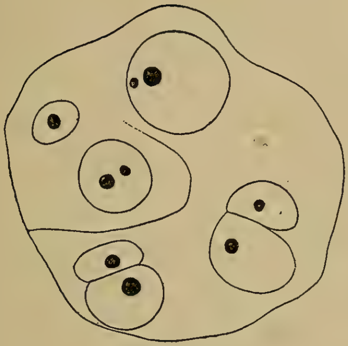
22



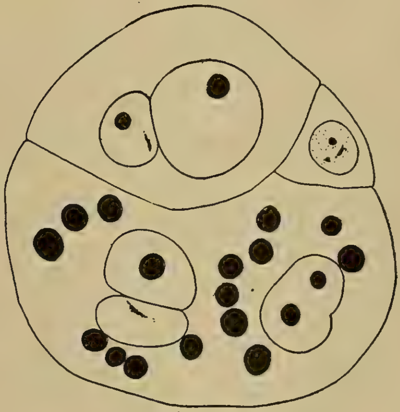
23



24



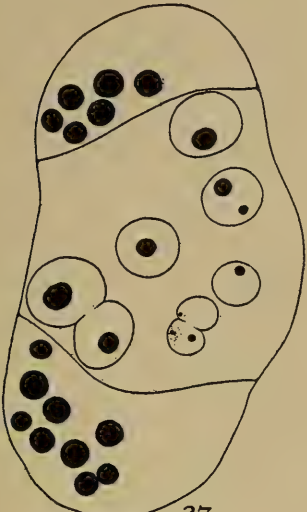
25

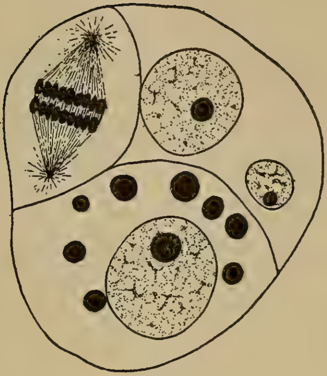


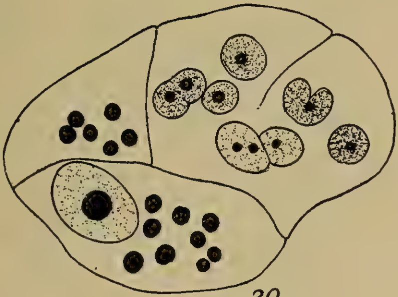
26

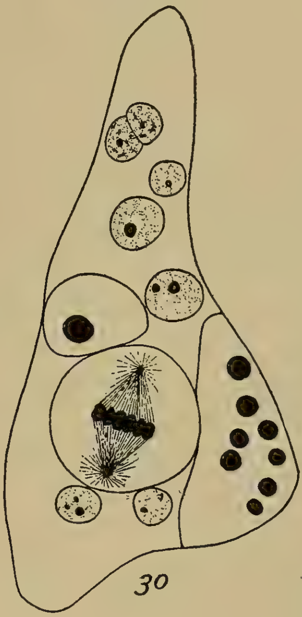
PLATE VI.

FIGS. 27–31. Later cleavage-stages.


27


28


29


30

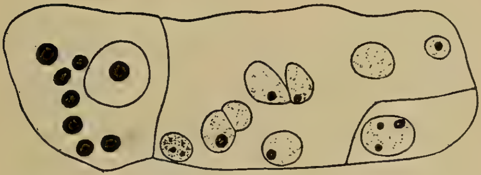
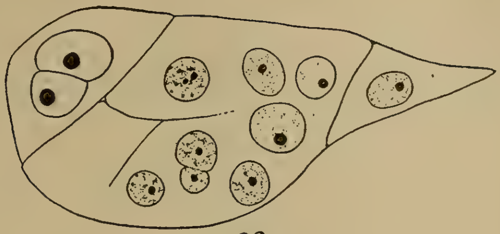
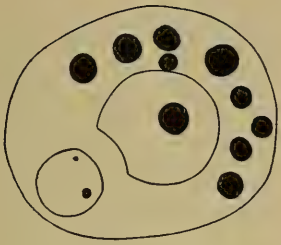

31

PLATE VII.

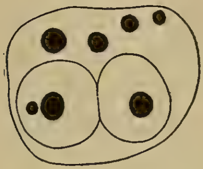
FIGS. 32–40. Cleavage-stages; all except Fig. 32 show single blastomeres or dividing nuclei.



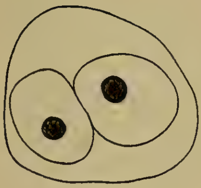
32



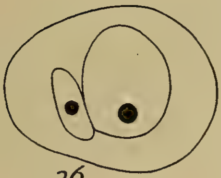
33



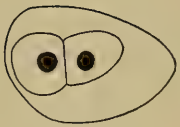
34



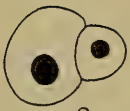
35



36



37



38

39

40

THE LONGEVITY OF MEMBERS OF THE DIFFERENT CASTES OF TERMOPSIS ANGUSTICOLLIS.

HAROLD HEATH.

Nearly five years have elapsed since I published an account[1] of the breeding habits of three species of California termites, and in this interval it has been possible to add a few facts relating chiefly to the age of the members of the various castes of *Termopsis angusticollis.* As was mentioned in the foregoing account, winged forms do not appear in colonies founded by a primary royal pair until the end of the second year though nymphs, that is larvæ with well developed wing buds, may be recognized be-before the close of the first year. Also in older colonies immature royal individuals, one or two molts removed from the adult condition, may be found in large numbers in nests from which the winged forms are ready to depart. As there is but one flight a year with this species, it follows that some of the members of the primary royalty are over one year of age before they leave the nest. Furthermore, I have in several instances removed from a flourishing colony a small band of soldiers and well developed workers, together with a number of young individuals which have not undergone more than two molts. The development of these last named insects may be followed without any particular difficulty and where they become true royal forms it is usually after they have been more than one year in the nest.

To determine the length of life of the true royal pair after their mating I partially buried a number of pine logs in a favorable situation and covered them with a cage constructed of fine wire netting. In it were placed, previous to the swarming season, a number of colonies housed in glass jars or naturally founded in logs which had been carried in from the fields. The escape of the winged insects from these nests was normal, and in a short time hundreds of royal pairs were engaged in constructing burrows, which, like the resulting colonies, were developed in the customary fashion as I determined from time to time. During the same period many naturally established nests were located, both at Pacific Grove and about Stanford University, and served to check up results.

---

[1] "The Habits of California Termites," BIOL. BULL., IV., 47-64.

In some instances the death of one or both of the royal pair, whether free or in captivity, took place before three years had elapsed; but in certain cases this was undoubtedly due to an unfavorable habitat occasioned by excessive drought or moisture or more often to the ravages of *Termes lucifugus.* Beyond this time their destruction could not so readily be traced to adverse conditions, and may rather be due to exhaustion produced by the arduous duties attendant upon the development of a healthy flourishing progeny. A careful examination showed that at the end of four and one half years the greater number of the kings and queens had died, not over 10 per cent. remaining. After five years I was able to find only six colonies, out of two hundred and thirteen, in which royal forms were present, and but two of these contained both king and queen. In a two-quart fruit jar, which was hermetically sealed and opened only once or twice a year to add water and wood and to remove portions of the continually increasing walls and barricades, I kept a royal pair a few days over five years and eight months. At this time the male died and the queen followed about three months later. From the foregoing it appears that the average life of the royal pair is of at least one year duration in their immature condition, and between four and five after leaving the nest. And further, the life of the male is of practically the same length as that of the female.

To determine the longevity of the workers and soldiers I have in several cases removed from a large colony one or two workers and soldiers which had recently undergone their final molt along with many smaller forms, and have thus been able to distinguish these larger insects from their fellows and to determine their span of life. And again, owing to some slight deformity or some mutilation it has been possible to recognize others in a normally developing colony and to trace their history for years at a time. Also I have taken large communities, headed by true or complemental royal forms, and by removing the young as fast as they appeared, have been able to determine the approximate length of life of all the individuals. From these observations it results that the workers live about four years after their final molt, and it is probable that this completed state is reached during a period of at least one year, so their life is terminated at the end of about five years.

The soldiers I have been unable to keep, in a great majority of cases, more than three years in a fully developed condition. In a few colonies I have kept them more than four years, and in the nest in the hermetically sealed jar two soldiers lived to be nearly five years of age. Generally speaking the average life of the soldier is about four years from the time of hatching.

While making these observations concerning the formation of the colony and its subsequent development, I have conducted a number of experiments to discover if possible the mode of formation of the various castes. A careful examination of the eggs and the newly hatched young fails to disclose differences which appear to be correlated in any way whatsoever with those distinguishing the soldier, worker and perfect insect to which they give rise. Grassi and Sandias,[1] and others with whom I agree, are of the opinion that it is a question of nutrition ; that the royal pair or the workers by judicious feeding direct the course of development along particular lines. I believe that theoretically all of the young are destined to become perfect insects, but in many cases their growth is arrested or modified and complemental royal forms or soldiers or workers result. Under certain circumstances the modification is not perfect and monstrous forms result, such as soldiers with wings and the ability to produce eggs which may develop. In the examination of many hundreds of colonies formed originally under natural conditions I have found a few such monstrosities, and the surroundings invariably suggest that they have developed under unnatural conditions. In most cases they have appeared in small fragments of wood, which have broken off from the main trunk inhabited by an extensive colony invariably headed by complemental royal forms. With the separation of a small portion of a community changed conditions must arise. New complemental royal forms and in certain cases additional soldiers have to be produced, and it is reasonable to suppose that the enlargement of the nest and the care of the eggs and the developing young demand a profound readjustment of the conditions obtaining under the old regime. It is accordingly not difficult to imagine that during this transition period the usual mode of feeding of certain individuals may be interfered with and unusual structures result.

---

[1] "Costituzione e Sviluppo della Società dei Termitidi," Catania, 1893, 150 pp.

In every case these unnatural types are imperfectly developed. For example, the wings of the soldiers are, so far as my experience goes, of less than average size, being in all but two cases less than the length of the abdomen. The reproductive organs likewise are imperfect. In one specimen one half of the ovary was partially functional, the other being in an abortive condition. In two other examples there was an incomplete development of both sides. The mandibles also are of less than average size though within the range of variability which Dr. Desneux writes me is extraordinarily great.

While appearances suggest that these unusual individuals are produced as a result of disturbed conditions, chiefly if not altogether connected with the food and method of feeding, there is no definite proof to show that such is actually the case. For several years I have carried on feeding experiments in the hope that some light might be shed on this important problem, but up to the present time the results are purely negative. I have fed hundreds of immature individuals, which had not undergone more than two molts, entirely or in part upon material carefully removed from the stomach of worker termites and in some instances mixed with fragments of the salivary glands, but such a diet is not perfect, or at all events the mode of feeding is too crude, for the insects soon die and cleared mounts show the alimentary canal to be practically empty. Older individuals live for a longer time on such fare but finally they likewise grow feeble and die. At other times I have fed young and old termites on proctodeal food but the results are never positive. Again I have added to sawdust, upon which these insects thrive, varying quantities of different salts, especially those used in experiments on chemical fertilization, and in other cases have used different acids of various strengths. In other experiments I have mixed the fragmented wood with many substances, nutritious and innutritious intended to disturb the nutritive processes in some degree, but here too, the animal may flourish or starve yet otherwise exhibit no unusual modifications.

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

VOL. XIII SEPTEMBER, 1907 NO. 4

CONTENTS

PAGE

CHILD, C. M. *Studies on the Relation between Amitosis and Mitosis, IV–VI.* 165

WHEELER, WILLIAM M. *On Certain Modified Hairs Peculiar to the Ants of Arid Regions* 185

BURNETT, THEO. C. *Can Sea Water Maintain the Beat of the Heart of Fresh Water Animals?* 203

STARKS, EDWIN CHAPIN. *On the Relationship of the Fishes of the Family Siganidæ* 211

CALKINS, GARY N. *The Fertilization of Amœba proteus* 219

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY
PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN	AGENT FOR GERMANY	AGENT FOR FRANCE
WILLIAM WESLEY & SON	R. FRIEDLÄNDER & SOHN	LIBRAIRIE ALBERT SCHULZ
28 Essex Street, Strand *London, W. C.*	*Berlin, N. W.* *Carlstrasse, 11*	*3 Place de la Sorbonne* *Paris, France*

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Smithsonian Institution
National Museum

Entered October 10, 1902, at Lancaster, Pa., as second-class matter,
under Act of Congress of July 16, 1894.

Editorial Staff.

E. G. Conklin—*The University of Pennsylvania.*

Jacques Loeb—*The University of California.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*American Museum of Natural History, New York.*

C. O. Whitman—*The University of Chicago.*

E. B. Wilson—*Columbia University.*

Managing Editor.

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Holl, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa.

Vol. XIII. *September, 1907.* *No. 4*

BIOLOGICAL BULLETIN

STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS.

IV. NUCLEAR DIVISION IN THE SOMATIC STRUCTURES OF THE PROGLOTTIDS OF MONIEZIA.

V. GENERAL DISCUSSION AND CONCLUSIONS CONCERNING AMITOSIS AND MITOSIS IN MONIEZIA.

C. M. CHILD.

IV. NUCLEAR DIVISION IN THE SOMATIC STRUCTURES OF THE PROGLOTTID OF MONIEZIA.

1. *The Genital Ducts.*

The various genital ducts of the cestode proglottid appear to arise by the division and differentiation of the parenchymal cells, if the syncytium which composes the parenchyma can properly be said to be composed of cells.

Moreover, this division and differentiation apparently occurs *in situ*, at least to a large extent, and not by outgrowth of the cells from a particular region. As was noted in the first paper of this series the development of the reproductive organs begins, or at least first becomes visible, in a region near the longitudinal nephridial canals and the lateral nerve cords, *i. e.*, midway between the region where the ovaries will appear and the lateral border of the proglottid (Child, '07*a*, Figs. 1*a*, 1*b*), the first portion to appear being parts of the ducts. The earliest indication of development is an increase in the number of the parenchymal nuclei in this region (Child, '07*a*, Figs. 2 and 3). The area of proliferation gradually extends in both directions and the "cells" differentiate into the ducts and terminal organs. The development of the ducts does not appear to be an outgrowth from the region first involved, but seems rather to be due to an extension of certain stimuli or conditions in the parenchyma which bring about proliferation and gradual differentiation of all cells which lie within the region affected. I have found it impossible to reach any other conclusion regarding the development of these ducts. Moreover, in the earliest stage of the ducts I have never observed a single case of mitosis or anything resembling it. On the other hand Figs. 2 and 3 of my earlier paper (Child, '07*a*) show that amitoses are frequent. In the early stages there is a marked difference in size of the nuclei in the central and peripheral portions of the area in which proliferation is occurring. This is very clearly shown in Fig. 2 (Child, '07*a*). Here the nuclei about the periphery of the proliferating region are of the same size as the nuclei elsewhere in the parenchyma, but toward the center their size decreases until they are only a small fraction of the size of the parenchymal nuclei in general. Evidently proliferation is much more rapid in the central than in the peripheral regions of the proliferating area.

In somewhat later stages the rapidity of division apparently decreases and the nuclei of the central regions gradually increase in size until they are almost or quite as large as those about the periphery. At this time the ducts are visible as bands or cords of very numerous nuclei each surrounded by small areas of cytoplasm. Between the ducts and other portions of the parenchyma, however, no limiting membrane exists nor do the "cells" appear appreciably different in character from those composing the parenchyma.

From this stage on the differentiation of the walls of the ducts gradually takes place; muscle-fibers develop, a lumen appears, and nuclear division becomes less and less frequent.

In Figs. 1–5 (Plates VIII. and IX.) typical portions of the duct-regions before differentiation has begun are shown. Fig. 1 (Pl. VIII.) is taken from the lateral end of the developing ducts at a time when the proliferation has extended to a point midway between the longitudinal nephridial canals and the lateral margin of the proglottid. From right to left the figure includes the whole width of the proliferating area from which this portion of the ducts will develop. The axis of the developing duct corresponds to a line drawn vertically through the middle of the figure. The nuclei are just beginning to be affected by the conditions which produce proliferation ; thus the figure represents approximately the earliest stage at which this portion of the ducts is distinctly visible. From the cord of cells in this lateral region both vagina and vas deferens differentiate, but there is no distinction between them until much later stages.

Fig. 2 (Pl. VIII.) represents a section similar to Fig. 1 of the cord of cells from the same region at a somewhat later stage. The nuclei are more numerous and amitoses are apparently more frequent. At the right is one of the very rare cases of mitosis observed in the development of the genital ducts. It will be observed that it lies on the border of the region involved in proliferation and the same is true of most other cases observed. Not a single case of mitosis has been observed in the axial region of the cell-cords from which the ducts arise and only six cases of mitosis have ever been seen outside the ovaries and testes in *Moniezia expansa* and *M. planissima*, though extended search for them has been made.

Fig. 3 (Pl. VIII.) is a section similar to Fig. 1 of the cord of cells from which the vas deferens develops, taken from a point near its upper or inner end where it does not adjoin the vagina. As regards nuclear division this figure is very similar to the preceding. On the right is shown one of the cases mentioned and figured in earlier papers (Child, '07*a*, '07*b*, '07*d*) where the two parts of a nucleus which is apparently undergoing amitosis stain differently.

In Fig. 4 (Pl. IX.) a transverse section of the vas deferens at a somewhat later stage after the lumen has appeared is shown. Amitotic nuclear divisions are still taking place, but apparently less rapidly than in earlier stages, or else the nuclei increase in size more rapidly between successive divisions.

Fig. 5 shows a portion of a vas efferens near its junction with others. Two cases of undoubted amitosis are visible. Fig. 6 is from the outer portion of the wall of the oviduct and includes one case of mitosis besides several amitoses.

The preceding figures are sufficient to indicate the general type of development of the genital ducts as regards division. Since we are concerned with the cytology rather than with the morphology, it is unnecessary to follow the development of the structures step by step, for the later stages show nothing of importance cytologically, which cannot be observed in the earlier stages. Amitosis continues to be the characteristic method of nuclear division throughout the development.

2. *Other Proglottidal Structures.*

The remaining figures (Figs. 7–19) are selected as examples from a large number of camera drawings of parenchymal structures.

Fig. 7 (Pl. IX.) probably represents one of the smaller nephridial ducts at an early stage in transverse section. One very clear case of amitosis and two other probable cases are visible.

Fig. 8 (Pl. IX.) and Fig. 9 (Pl. X.) are taken from the region of the lateral longitudinal nerve-cords in the growing region just posterior to the scolex. In this part of the body the cytoplasmic areas about the nuclei are more extensive than further posteriorly.

Figs. 10–14 (Pl. X.) show cells and syncytial masses from the general parenchyma in the region where the proglottidal boundaries are just becoming visible, *i. e.*, in a region of rapid proliferation of the parenchymal nuclei. Not a single case of mitosis has ever been observed in this region.

Two cells from the interproglottidal glands are shown in Figs. 15 and 16 (Pl. X.) at a stage where differentiation is advanced. In early stages the regions where these glands appear are not visibly different from other regions. The first visible indication of gland-development is the formation by amitotic proliferation of groups of nuclei along the interproglottidal boundary. Each of these groups gives rise to a considerable number of elongated unicellular glands like those in the figures, arranged radially about a common outlet. Amitosis is much more frequent before differentiation occurs, but as Figs. 15 and 16 indicate, often occurs after differentiation is somewhat advanced. The figures show the earliest visible stages in the formation of the secretory product. The cytoplasm gradually becomes filled with deeply staining, granular masses.

Cuticle-forming cells of the external layer of the body are shown in Figs. 17–19. These cases are taken from the region where proglottids are beginning to form: in this region division is more frequent in these cells than in proglottids advanced in development.

It is perhaps unnecessary to repeat that these figures are merely a few cases selected almost at random from among my camera-drawings. The number of figures might be increased almost indefinitely if desirable, but I think these are sufficient to show that amitosis is a typical feature of development in *Moniezia* both in the germ cells and in the soma.

Unfortunately it is at present impossible to complete my observations by examination of the stages between the embryo and the adult tape-worm, for the life-history and intermediate host of the genus are unknown.

V. General Discussion and Conclusions Concerning Amitosis and Mitosis in Moniezia.

Before any general interpretation of the facts concerning the rôle and frequency of the two forms of division is possible it is necessary to know something regarding the physiological conditions which determine the occurrence of each of the two methods of division. At present, however, we have no real knowledge regarding these conditions and are limited to hypotheses and surmises. In a recent paper (Child, '07*c*) I have made certain suggestions along this line to which attention may be called here.

It was pointed out in the paper referred to that the stimulus to nuclear division and growth is apparently not identical with the presence of an excess of nutritive material, for it is a well known fact that extensive regeneration will occur in many forms, *e. g.*, *Planaria*, even after reduction of the body by long continued starvation, to a fraction of the original size. In such cases the regenerating region derives its nutritive material from other regions of the body, which consequently decrease in size more rapidly than in individuals where no regeneration is taking place. Evidently in such cases the nutritive material goes to the regenerating region at the expense of other parts because the demand is greater there than elsewhere. In short, conditions exist in this region in consequence of which it deprives other regions of material. Doubtless these conditions are largely chemical in nature.

The existence of such conditions in this and other similar cases justifies the conclusion that the stimulus to cell-division and growth is not identical with the presence of excess of nutritive material. Admitting the existence of a stimulus to division independent of the presence or absence of nutritive material we may expect to find conditions different within the cell or nucleus, according as the changes which occur in consequence of the stimulus are or are not balanced by the intake of material. In case they are balanced, a condition of equilibrium is more or less perfectly maintained or else changes in both directions from the condition of equilibrium alternate more or less rhythmically. In consequence of the complex character of the cell and the more or less central position of the nucleus, the occurrence of rhythmical cyclical changes is to be expected rather than the maintenance of a condition of absolute equilibrium.

If on the other hand the demands of the nucleus are not met by the intake of material the condition of equilibrium is not attained, and the processes in the cell, so far as this point is concerned, are not cyclical, but acyclical or orthodromic.

Now the nuclear phenomena which occur in connection with mitosis are very clearly cyclical; the condensation of the chromatin and the disappearance of the nuclear membrane are followed by an apparent reversal which terminates in the reconstitution of a nucleus and the resolution and distribution of the chromatin.

In amitosis, on the other hand, no such cyclical changes occur. The nuclear structure remains the same throughout the whole process. There is no disappearance or transformation, followed by return to the original condition, of any part. Apparently the process consists essentially in increase in size in consequence of formation of new nuclear material, followed by separation into physiologically independent parts. Nothing in the visible phenomena indicates the occurrence of reversal in direction of the processes involved. Division itself may be due either to physical factors or to the establishment with increasing size of more or less independent regions or centers of activity. The appearance of new nucleoli in widely separated regions of the nucleus and the difference in tingibility of the two parts of a dividing nucleus, which are often observed in *Moniezia*, indicate the existence of a certain degree of physiological independence before separation of the parts.

If this interpretation of the visible phenomena is correct it appears probable that mitosis is associated with certain cyclical processes, in the nucleus and amitosis with acyclical or orthodromic processes. In other words, in order to divide mitotically the nucleus must be in a condition approximating equilibrium between intake of material and functional transformation. If the stimulus to growth is so strong that the nucleus is forced far from a condition of equilibrium amitotic division may occur. Of course in all cases where the demand exceeds the supply the actual rate of growth or division is determined not by the intensity of the stimulus but by material available. According to my suggestions, it is not rapidity of growth or division which determines or influences the form of division, but rather the relation between the stimulus to growth and the intake of material. Division itself is probably an incidental result of growth. These suggestions are of course merely provisional, being scarcely more than surmises, but they may perhaps serve as a working hypothesis for future investigation of the problem.

But whatever value the future may assign to these suggestions, the facts now known regarding the occurrence of amitosis seem to be in accord with this hypothesis, as will be clear from a brief consideration.[1] Most of the earlier observers agree in the conclusion that amitosis is characteristic of regions of extreme physiological activity connected with assimilation, secretion, etc. (Ziegler, '91 ; Ziegler and Vom Rath, '91 ; Vom Rath, '95, etc.). More recent observations of others and myself some of which are briefly mentioned in my earlier paper (Child, '07*c*) show that amitosis is of frequent occurrence in regulatory growth, which is much more rapid than normal growth. It is also the typical form of division in the imaginal organogeny of certain diptera and very probably of many insects. Here, likewise, the growth is exceedingly rapid. In certain vertebrate embryos amitosis is apparently much more frequent in the more rapidly growing than in the less rapidly growing regions. In certain forms, such as trematodes and polychætes, which rapidly produce a very large number of generative cells, amitosis appears to be the characteristic method of division in the primitive germ-cells.

---

[1] More extended discussion of the bibliography is postponed until additional data have been recorded.

All of these cases concern regions of extreme assimilative activity and there are certain indications that, so far as the individual nuclei are concerned, they are regions which are far from equilibrium. In all such regions, for example, the cytoplasm is relatively small in amount, *i. e.*, the nuclei are relatively much more numerous than in less rapidly growing regions ; secondly the nuclei in these regions are usually much smaller than in less rapidly growing regions. Often there are differences in this respect between the peripheral and central portions of such regions, the central portions showing more extreme conditions than the peripheral. Evidently the nuclei in these regions do not attain a condition of equilibrium but are forced on in a given direction by some stimulus as rapidly as the material available will permit. According to the hypothesis set forth above these are exactly the conditions in which amitosis may be expected to occur.

But according to this hypothesis also amitosis is not necessarily confined to regions of rapid growth. If little material is available actual growth may be exceedingly slow. Moreover, it is possible that nutritive material may be present outside the cell or even in the cytoplasm, in excess but in consequence of scarcity of cytoplasm or other special conditions may become available for the nucleus only very slowly or not at all. In such cases amitosis might occur in the presence of an apparent excess of nutritive material. Possibly various cases of amitosis observed in nuclei lying in the yolk of meroblastic eggs and surrounded by only a small amount of cytoplasm may be cases of this kind.

And again it is possible that the differentiation of the cytoplasm in certain directions may bring about conditions favoring amitosis ; in such cases the amount of undifferentiated cytoplasm may be insufficient to maintain the nucleus in equilibrium. Thus amitosis may be expected, and has often been found in highly differentiated cells.

Doubtless various other conditions may arise in the cell which favor amitosis, but the above consideration is sufficient to indicate the relation between the facts and the hypothesis.

The opinion of Ziegler and Vom Rath (Ziegler, '91 ; Ziegler, and Vom Rath, '91 ; Vom Rath, '95) and many later observers, that amitosis is never followed by mitosis, but always leads to degeneration and death is without doubt incorrect. But that degeneration and death should follow amitosis in certain cases is to be expected, if my hypothesis is correct. Under extreme conditions the nucleus or cell many be forced so far from equilibrium that changes occur which render return impossible, and degeneration and death follow. In regions of rapid embryonic growth degenerating nuclei are not infrequently found, but in these, as in other cases, the degeneration is not a necessary consequence of the amitosis, but both are merely indications of a physiological condition, which in many cases brings about amitosis without degeneration, but in extreme cases produces degeneration. Nuclear fragmentation is a frequent accompaniment of degeneration, but even in these cases the physiological conditions may be in general similar to those occurring in normal amitosis.

Returning now to the case of *Moniezia*, the facts concerning the distribution of relative frequency of amitosis and mitosis are briefly as follows. As regards the germ cells, amitosis is much more frequent than mitosis during the development of ovaries and testes (Child, '07*a*, '07*b*). Mitoses are of very rare occurrence during the earlier stages of development, but in some chains and proglottids and gonads appear somewhat more frequently in later stages before the growth-period. This developmental period characterized by amitosis is followed by the growth-period, at the beginning of which a spireme appears, and later by maturation. In the female cells maturation exhibits the features typical of the process in other species (Child, '07*a*) : in the male cells typical maturation occurs, but in addition to this a peculiar process of fragmentation of the nuclei of the first spermatocytes is of common occurrence (Child, '07*b*), and apparently results in the formation of nuclei indistinguishable from the spermatid-nuclei formed in the typical manner. Whether these nuclei actually give rise to spermatozoa or not cannot be determined with certainty, but the observations seem to indicate that they may. The relative frequency of the two processes, typical maturation and fragmentation, appears to vary in different chains, proglottids, and regions. In some chains typical maturation has been observed only rarely, and fragmentation very frequently in the testes, yet these chains apparently produce as many spermatozoa as others; in some proglottids of certain chains the maturation-phenomena are similarly very infrequent while fragmentation is of common occurrence, while in other proglottids typical maturation is more frequent, and finally, in many proglottids the maturation-phenomena have been observed much more frequently in those testes which occupy the lateral regions of the proglottid, while fragmentation appears to be more characteristic of the testes in the middle regions.

The first cleavage of the egg is at least usually, if not always mitotic, but in later stages amitosis becomes the characteristic method of division, mitosis appearing only occasionally, and then in the larger nuclei (Child, '07*d*). Throughout the stages of cleavage observed nuclear division is far in advance of cytoplasmic division.

In the development of somatic structures in the proglottid mitosis is almost never seen, amitosis being the typical method of division. When mitoses occur they occur as isolated cases, usually at or near the periphery of regions of proliferation.

If my observations are correct, amitosis is the more common method of division in the generative cycle, except during the period of maturation and early cleavage. In the somatic cells of the adult body it appears to be the usual method at all times. Later embryonic stages inhabiting the intermediate host are not at present known, but conditions will probably be found to be similar in these.

Considering these facts in the light of the hypothesis presented above, we find them in general in accord. In the first place *Moniezia* produces rapidly an enormous number of generative cells and this involves a very large amount of assimilation. These, according to our hypothesis, are conditions favorable to the occurrence of amitosis, and we have found that amitosis is the characteristic method of division in the development of ovaries and testes.

But even in this period mitoses occasionally occur, their relative frequency differing in different chains, proglottids and regions. These differences may be due to local differences in nutrition; doubtless different chains and often different proglottids, receive different amounts of nutritive material, and in those best nourished, some of the nuclei may attain equilibrium occasionally. The fact that mitoses were more frequently observed near the lateral borders than in the middle of the proglottid may be due to similar differences in condition. Near the lateral border the proliferating regions — chiefly testes — are less numerous than in the middle regions and the absorptive surface is relatively greater hence more nutritive material may be available for each, and conditions permitting mitosis may be more frequently attained than elsewhere.

Moreover, mitoses in the developing testes and ovaries seem to occur more frequently in the later stages during the last division preceding the growth period, than in earlier stages. It is probable that in these stages the stimulus to growth is not as great as in earlier stages and some of the nuclei attain a condition of equilibrium.

It is by no means certain that the peculiar process of fragmentation of the spermatocyte-nuclei (Child, '07*b*) is to be regarded as due to the same conditions as other cases of amitosis. On the other hand, there can be little doubt that the conditions which, according to the hypothesis, favor amitosis are present in the testes. The development of each testis involves the formation of a relatively enormous amount of nuclear and cytoplasmic material and each prologottid contains hundreds of testes. Failure to attain equilibrium might be expected here if anywhere. Indeed the frequent degeneration of masses of cells in the testes (Child, '07*b*) seems to indicate very clearly that insufficiency of nutrition and failure to attain equilibrium exist, especially as this degeneration is much more frequent in some chains and proglottids than in others. Apparently some cells are forced so far from equilibrium that they can no longer exist. It has been noted (Child, '07*b*) that such degeneration of cells was not observed in stages from the beginning of the growth-period to the spermatid. It is not improbable that cells which once enter upon the growth-period possess sufficient energy to obtain nutritive material, notwithstanding the demands of their rivals in earlier stages, but it is also possible that some of these cells are so far from a condition of equilibrium that they cannot go through the maturation mitoses following the growth-period. Such cells are probably those in which fragmentation occurs. Certainly the process of fragmentation in the spermatocytes (Child, '07*b*) presents no difficulties to such an interpretation. In it most of the nuclear substance disappears and a few small nuclei are formed from what might almost be regarded as the debris of the original nucleus. Apparently the old nucleus is no longer able to exist as a physiological system and small parts of it form new systems. The special form of fragmentation in these cases may be merely the result of special conditions which are not present in the primitive cells of earlier stages.

It is evident then that these cases of fragmentation in the spermatocytes can readily be interpreted in the same manner as the amitoses in other stages. Whether the resulting nuclei actually take part in the formation of spermatozoa is a question which cannot be decided at present.

The typical spermatocytic mitoses were observed more frequently in testes situated in the extreme lateral regions of the proglottids, fragmentation apparently being much more frequent in the middle regions (Child, '07*b*). This fact likewise is probably to be regarded as favoring the suggestions made above. As was pointed out, in the extreme lateral regions of the proglottid the testes are not as numerous as in the middle regions and the resorptive surfaces from which nutritive material may reach them are relatively greater than in the middle regions. It may well be that the cells in these testes attain more frequently than others a condition in which mitosis is possible.

As regards the frequency of amitosis in cleavage of the earliest stages the fact that nuclear division is always far in advance of cytoplasmic division, except in those blastomeres which divide mitotically (Child, '07*d*) seems to indicate the existence of a strong stimulus to nuclear division in the nuclei dividing amitotically. Apparently most of the nuclei are forced by some factor to divide much more rapidly than they acquire correlations with the surrounding cytoplasm, and so do not attain a condition in which the cyclical processes characteristic of mitosis can occur. It is not possible to determine the nutritive conditions with any certainty at this stage. It is very evident in the later stages, however, that those regions of the egg where the nuclei are smallest and amitosis is most frequent are regions from which the yolk is absent (Child, '07*d*, Figs. 27, 29, 30, 31). In the yolk-bearing regions the nuclei divide much less frequently and more often mitotically than in other regions. These facts point in the same direction as those already cited with respect to other stages.

In the somatic structures of the proglottid, mitosis has never been observed except in the lateral regions of the proglottid and very rarely there. When mitosis has been observed in the development of the genital ducts it was usually at or near the periphery of the proliferating region, in one of the cells which was evidently less intimately involved in the proliferation than those nearer the middle. These facts are in line with the preceding. Moreover the somatic cells usually possess only a very small amount of undifferentiated cytoplasm and this may be a factor in determining the physiological condition of the nuclei and so the form of division.

It would appear then that the facts concerning occurrence and relative frequency of amitosis and mitosis in *Moniezia*, as well as in other forms, do not conflict with the suggestions made by way of interpretation. There can I think be little doubt that the two forms of division correspond to different physiological conditions in the nucleus. Judging from the visible phenomena, it also seems probable that mitosis is associated with cyclical, and amitosis with acyclical processes. The questions as to the availability of nutritive material and "equilibrium" are more obscure and complex, for they concern not merely the presence or absence of nutritive material outside of the cell, but, and probably chiefly, its availability within the cell and for the nucleus. Various factors, such as quantity and quality of cytoplasm, size, form, and condition of nucleus, etc., may conceivably play a part in determining the physiological conditions in the cell. Moreover, we know little regarding the nature of nuclear equilibrium. I have used the term merely with reference to a condition in which cyclical processes with periodical reversals occur.

Doubtless, also, there are characteristic differences in different species. In the life-history of *Moniezia*, for example, rapid and enormous growth is a characteristic feature. In such a form the stimulus to division and growth must be more powerful or else the nuclei must react more readily than in other species in which the life-history does not involve such extensive growth. Whichever the case, we may expect to find the acyclical nuclear phenomena more characteristic of the species with extensive or indefinite growth than of others, simply because in the former the amount of synthesis is much greater and relatively more rapid than in the latter. If amitosis is associated with these extreme assimilative conditions, as our hypothesis postulates, then its distribution and frequency will follow the same rule.

Similar differences may be expected in different organs and regions of the individual, and so far as our knowledge goes at present, they appear to exist. Amitosis seems to be more characteristic of rapidly growing or assimilating organs and regions than of other regions.

If these observations and suggestions are correct, we can no longer regard mitotic figures as the sole criterion of nuclear division in organisms. In many forms, such, for example, as *Planaria*, in mid-summer, when growth and nuclear division are very rapid and fission is occurring every few days, mitoses are rarely seen, but amitoses are very abundant. In various cases of form-regulation, which were formerly supposed to occur without cell-division because no mitoses were observed, amitosis is very frequent.

Furthermore, if amitosis may occur in the normal developmental cycle and if it is especially characteristic of regions of rapid growth, as the facts indicate, we cannot depend upon the distribution of mitotic figures in developing tissues as an indication of the rapidity of cell-division and growth in different regions. The regions where mitoses are most abundant may be the regions of slowest division instead of the only regions where division is occurring.

The bearing of these and other observations on certain cytological hypotheses is briefly discussed in another paper (Child, '07*c*) and requires no further consideration here. I hope in future to continue these observations on other species and to offer further evidence for or against the hypothesis presented here, together with a more extended discussion of the observations of others.

April, 1907.

BIBLIOGRAPHY.

Child, C. M.

'07a Studies on the Relation between Amitosis and Mitosis. I. Development of the Ovaries and Oögenesis in Moniezia. Biol. Bull., Vol. XII., No. 2, 1907.

'07b Studies on the Relation between Amitosis and Mitosis. II. Development of the Testes and Spermatogenesis in Moniezia. Biol. Bull., Vol. XII., Nos. 3 and 4, 1907.

'07c Amitosis as a Factor in Normal and Regulatory Growth. Anat. Anz., Bd. XXX., Nos. 11 and 12, 1907.

'07d Studies on the Relation between Amitosis and Mitosis. III. Maturation, Fertilization, and Cleavage in Moniezia. Biol. Bull., Vol. XIII., No. 3, 1907.

Vom Rath, O.

'95 Ueber den feineren Bau der Drüsenzellen des Kopfes von *Anilocra mediterranea* Leach im Speciellen und die Amitosenfrage im Allgemeinen. Zeitschr. f. wiss. Zool., LX., 1895.

Ziegler, H. E.

'91 Die biologische Bedeutung der amitotischen (direkten) Kernteilung im Tierreich. Biol. Centralbl., Bd. XI., No. 12, 1891.

Ziegler, H. E., and O. Vom Rath.

'91 Die amitotische Kerntheilung bei den Arthropoden. Biol. Centralbl., Bd. XI., No. 24, 1891.

EXPLANATION OF PLATE VIII.

FIG. 1. A very early stage of the developing genital ducts in the region lateral to the longitudinal nephridial canals. The width of the figure from right to left represents approximately the width of the region involved in proliferation and the axis of the duct corresponds with a line through the middle of the figure from top to bottom

FIG. 2. A somewhat later stage in the development of the genital ducts, from the same region and showing one case of mitosis. Plane of section as in Fig. 1.

FIG. 3. The developing vas deferens near its inner end. The width of the figure represents the width of the proliferating region. Plane of section as in Fig. 1.

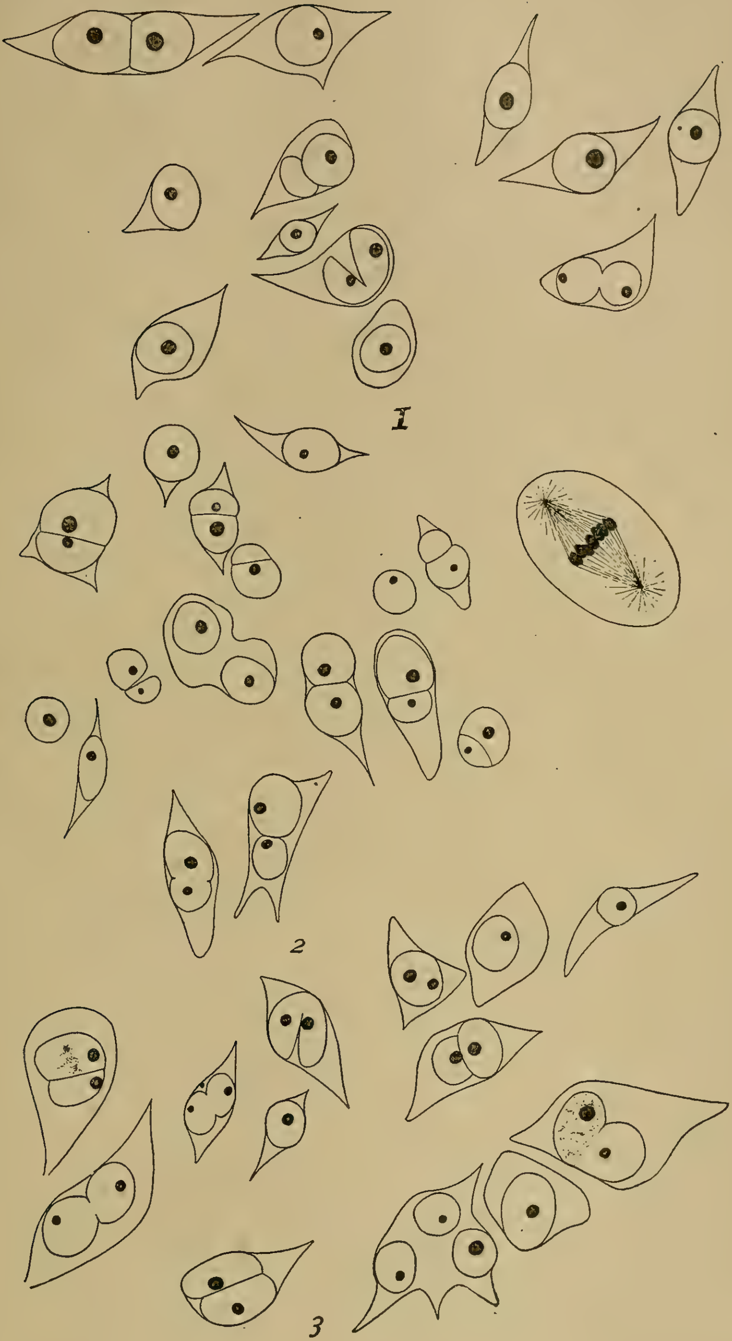

1
2
3

PLATE IX.

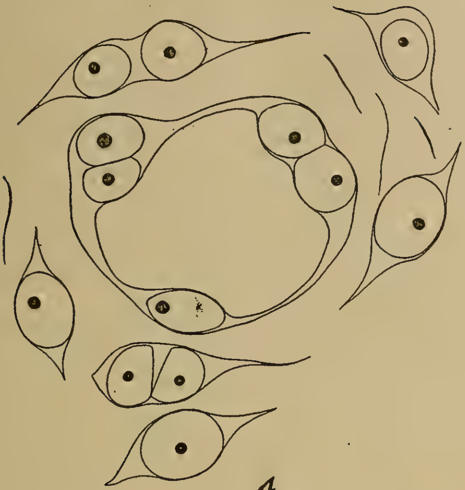
FIG. 4. Cross-section of the vas deferens at a somewhat later stage.

FIG. 5. Longitudinal section of a vas efferens.

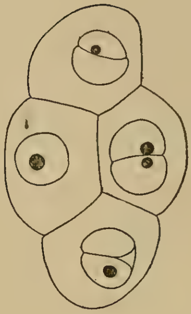
FIG. 6. A portion of the wall of the developing oviduct, showing one case of mitosis.

FIG. 7. Probably a developing nephridial duct in cross-section.

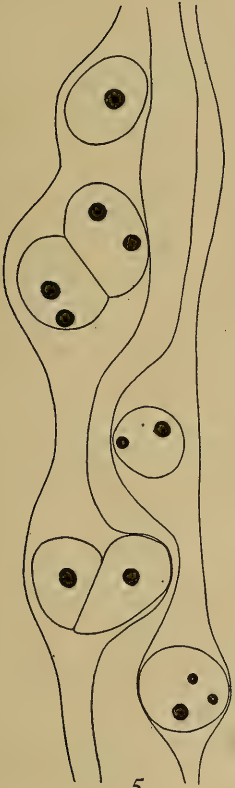
FIG. 8. From the region of the lateral longitudinal nerve-cords in the "neck."



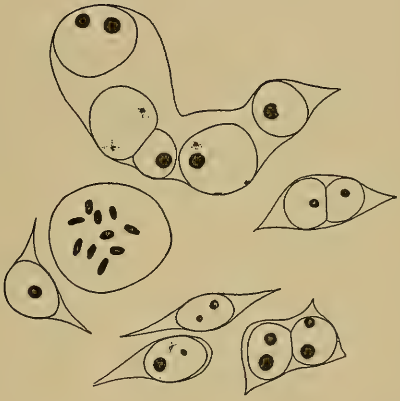
4



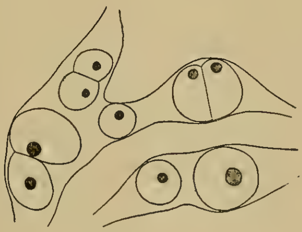
7



5



6



8

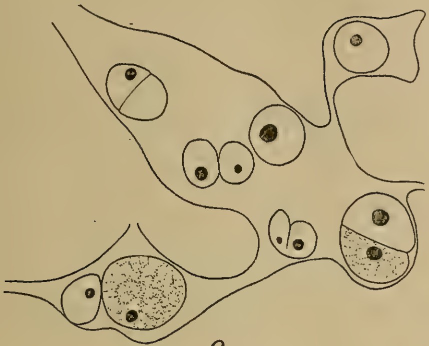
Plate X.

Fig. 9. From the region of the lateral longitudinal nerve-cords in the "neck."

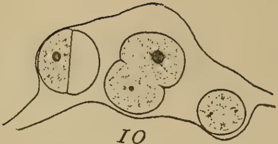
Figs. 10–14. From the general parenchyma in neck-region.

Figs. 15–16. Interproglottidal glands after differentiation has begun.

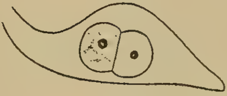
Figs. 17, 18, 19. Cells of the subcuticular layer from the neck-region.



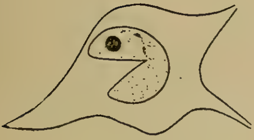
9



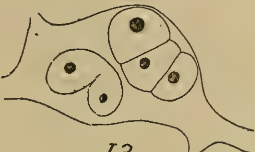
10



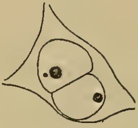
11



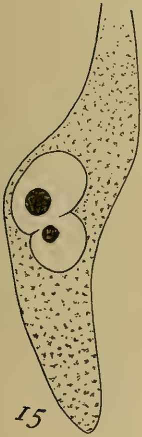
12



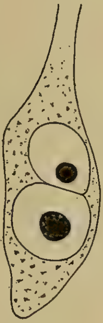
13



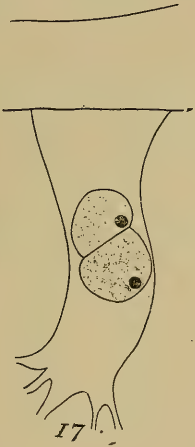
14



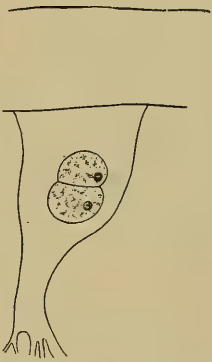
15



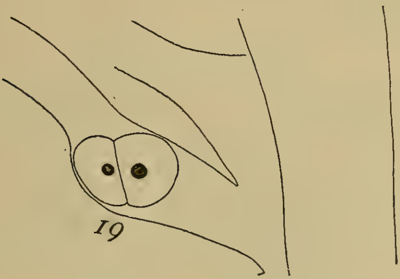
16



17



18



19

ON CERTAIN MODIFIED HAIRS PECULIAR TO THE ANTS OF ARID REGIONS.

WILLIAM MORTON WHEELER.

While studying the ants of the southwestern States I have been impressed with the series of unusually long hairs, or macrochætæ, which occur on the lower surface of the head and mandibles and on the anterior border of the clypeus in the workers and females of several species peculiar to the arid plains and deserts. Examination of the ants from similar localities in South America and from the still more arid regions of Africa, shows a prevalence of the same structures. There is also observable a tendency for these hairs to reach their greatest development in the species inhabiting the driest deserts. Although these structures have long been known to descriptive myrmecologists, no one, to my knowledge, has noticed the connection between their presence and a pronounced xerophily, or has endeavored to ascertain their function. These matters are of some interest, because the macrochætæ occur in several unrelated genera belonging to three of the five subfamilies of the Formicidæ, and therefore represent a striking example of convergent development. It is, furthermore, comparatively easy to account for the structures in question, as they are merely elongated and somewhat modified portions of the general hairy investiture of the ant's body.

Before treating of the function of these hairs and their occurrence in the various genera and subgenera, it will be advisable to give a general account of their arrangement. When most completely developed, they may be said to constitute four series, two paired and two unpaired as follows :

1. *Clypeal Macrochætæ.* — Although many ants have a fringe of long hairs or bristles on the anterior border of the clypeus, these are best developed in the desert species. They are curved downwards at their tips, are longest and most projecting on the free median portion of the clypeal border, and gradually grow shorter towards the lateral corners.

2. *Mandibular Macrochætæ.* — Both the dorsal and ventral surfaces of the mandibles in many ants are clothed with short, more or less projecting hairs, but the xerophilous species have in addition on each of the jaws, a ventral series of long and rather slender macrochætæ, which project downward and have their tips curved inward and sometimes upward. These hairs are longest on the bases and gradually become shorter towards the tips of the mandibles.

3. *Mental Macrochætæ.* — Some of the xerophilous species have a tuft of macrochætæ on the postero-median portion of the mentum, just in front of the anterior border of the gula. In one genus (*Ocymyrmex*) they extend backward and downward, in two others (*Myrmecocystus, Melophorus*) they project forward; in all cases their tips are turned forward or upward.

4. *Gular Macrochætæ.* — In most of the xerophilous species, these constitute the longest and most conspicuous hairs on the whole body. They are inserted, often in an arcuate series, on the posterior or lateral portions of the gula, are directed forward and downward and are often curved upward at their tips.[1]

As these various series of hairs or bristles together constitute a circumoral system, one is naturally inclined, on inquiring into their function, to suspect that there may be a connection between their development and some peculiarity in the feeding habits. It is true, to be sure, that these habits are apt to be highly specialized in desert ants. The natural and primitive food of ants consists of insects or the juices of plants, the latter collected either directly from the floral and extrafloral nectaries or indirectly after passing through the bodies of phytophthorous Homoptera (Aphididæ, Coccidæ, Membracidæ). But as insects and flowers are rare for long periods of the year in arid regions, many xerophilous ants have taken to harvesting and eating seeds. Others, like the honey ants (American species of *Myrmecocystus*, Australian *Camponotus* and *Melophorus*), still collect plant juices with avidity, but as such liquids are scarce or only temporarily abundant, they store them in the distensible crops of certain workers, which thus function as living bottles (repletes, or plerergates). Still other ants, like many *Myrmecocysti*, both in the Sahara and in the deserts of the southwestern States, have exaggerated the primitive entomophagous habit and have become very agile, predatory hunters.

---

[1] The term *gula* is here used in the sense of Janet ("Recherches sur l'Anatomie de la Fourmi et Essai sur la Constitution Morphologique de la Tête de l'Insecte," Paris, G. Carré and C. Naud, 1900, 205 pp., 15 Pls.) as that portion of the cranial capsule which arises from the fused labial, maxillary and mandibular segments. It comprises the greater portion of the heavily chitinized antero-ventral integument of the head, and is divided into two parts by a median longitudinal suture, the external indication of the gular apodeme. In reality, as Janet has shown, the gular apodeme represents the whole labial and maxillary and the mesial portion of the mandibular segment of the cranium, so that the gula proper comprises not only the lateral, but also the larger portion of the mandibular segment.

As the circumoral macrochætæ occur in desert ants that have specialized in each of these three directions, it is difficult to detect any relation between the development of such structures and the character of the food. But inasmuch as the food of all ants really consists exclusively of liquids either imbibed directly or carefully expressed from moist solids, I was led at first to adopt the following hypothesis: When the mandibles are wholly or partially closed, the macrochætæ are seen to form a crate or lattice-work, enclosing a lenticular space on the ventral side of the head. A drop of liquid carefully introduced into this space by means of a fine pipette will fill it and hang securely suspended from the flat or concave gula, with the spherical surface supported by the hairs of the crate. This experiment led me to the opinion that the hairs might be used for one or both of two purposes: first, to retain regurgitated drops of liquid and prevent their falling to the earth while the ants are feeding their larvæ or one another; and second, to enable the ants to collect from the stones and desert plants drops of rain water and to carry these to their nests. The hairs would certainly seem to be admirably adapted to both of these liquid-saving functions. This hypothesis seemed to be confirmed by the following observation on our northern *Stenamma* (*Aphœnogaster*) *fulvum*, published by Miss Fielde:[1] "I have observed that these ants, like the termites, are able to carry water for domestic purposes. They probably lap the water into the pouch above the lower lip and eject it at its destination. A hundred or two ants that I brought in and left in a heap of dry earth upon a Lubbock nest, during the ensuing night took water from the surrounding moat, moistened a full pint of the earth, built therein a proper nest, and were busy depositing their larvæ in its recesses when I saw them on the following morning." Miss Fielde assures me that she has repeatedly observed this interesting occurrence, especially when the ants had larvæ or pupæ, to which contact with perfectly dry earth would, of course, soon be fatal. There is, therefore, nothing extravagant about the view that desert ants, living in very dry soil, might carry water in the macrochætal crate instead of in the crop or hypopharyngeal pocket.

---

[1] "Further Study of an Ant," *Proc. Acad. Nat. Sci. Phila.*, 1901, pp. 521–544.

In order to ascertain, if possible, the true state of affairs from the ants themselves, I requested Miss Augusta Rucker to send me a number of living workers of the Texan harvester (*Pogonomyrmex barbatus* F. Smith var. *molefaciens* Buckley), a species with well-developed circumoral macrochætæ. The study of these insects in an artificial nest soon convinced me that my hypothesis, at least so far as this form is concerned, was erroneous. Though the ants were kept for several days without water and then given the liquid in small drops on the floor of their nest in imitation of the drops left by a shower on the stones and plants around the nests in their native environment, they were never seen to take it up into the macrochætal crate but simply lapped it up with their tongues. Protracted observation also proved that these ants never feed one another by regurgitation but that each worker partakes individually of the seeds, sugar, insects,

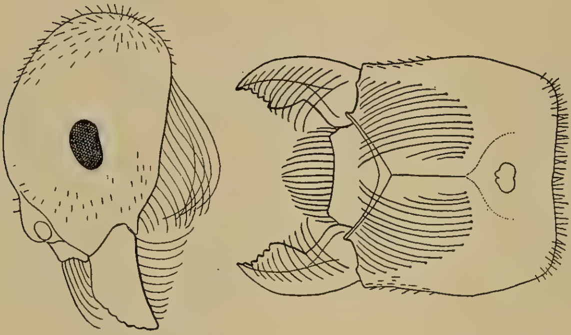


FIG. 1. *Pogonomyrmex barbatus* F. Sm.

etc., brought into the nest. I had previously found that this species does not feed its larvæ by regurgitation but with pieces of seeds or insects.

Continuing my observation of the living harvesters I was soon led to what I believe to be the true function of the macrochætæ. Each of the fore legs, as in other Formicidæ, bears a well-developed strigil, or enlarged and pectinated spur (Fig. 2) which in the living insect is usually carried at an angle of about 70° with the long axis of the tibia. The tibia is furnished at its tip on the mesial side with a dense brush of blunt hairs and the metatarsus is curved outward at the base, with a regular comb of fine bristles at the concavity and a series of coarser and blunter bristles along its whole mesial border. The pectinated strigil may be opposed to the pectinated and concave base of the metatarsus, and is especially adapted for cleaning the antennæ, which are drawn through the orifice between the two combs.

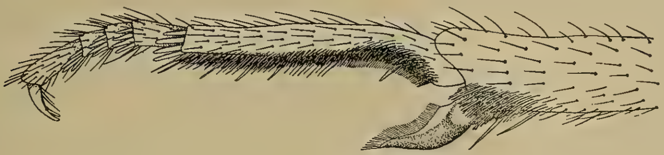


FIG. 2. *Pogonomyrmex barbatus*, fore tarsus, mesial side.

McCook[1] has described and figured (Pl. XIV., Fig. 64) the fore leg of *P. molefaciens*. I quote the following from his detailed account of the toilet habits (pp. 127–129): "The ants engaged in cleaning their own bodies have various modes of operating. The fore legs are drawn between the mandibles, and, so far as could be ascertained, also through or along the lips, and then are passed alternately back of the head, over and down the forehead and face, by a motion which closely resembles that of a cat when cleansing with its paw the corresponding part of her head. Sometimes but one side of the head is cleansed, in which case the foot used is drawn through the mandibles or across the teeth of one mandible after every two or three strokes upon the face.

---

[1] "The Natural History of the Agricultural Ant of Texas, a Monograph of the Habits, Architecture and Structure of *Pogonomyrmex barbatus*." Author's edition. *Acad. Nat. Sci. Phila.*, 1879.

These strokes are always made downward, following thus the direction of the hairs." . . . "Not only the fore pair, but also the other legs are passed — as above described — through the mouth. The second and third pairs are also and oftener cleansed by the fore legs, as follows: The ant throws herself over upon her side, draws up the middle and hind legs, which are interlocked at the tarsi, and then clasping them with one fore leg, presses the other downward along the other two. The fore legs alternate in this motion. When the legs on one side are cleansed, the ant reverses her position and repeats the process. When the antennæ are cleansed they appear to be taken between the curved spur at the extremity of the tibia and the tibia itself, as one would clasp an object between the base of the thumb and the hand, and are drawn along toward the tip of the flagellum evidently with one pressure." The cleansing of the abdomen or gaster is described as follows: "The hind legs are thrown backward and well extended, the middle pair nearly straight outward from the thorax, and less extended, so that the body is able to assume a nearly erect posture. The abdomen is then turned under the body and upward toward the head, which is at the same time bent over and downward. The body of the ant thus forms a letter C, or nearly a circle. The fore feet have meanwhile clasped the abdomen, and the work of brushing has begun. The strokes are directed upward toward the apex of the abdomen, and the foot passes around and beneath the under part, which is now toward the sternum, the apex is frequently licked by the tongue, and the feet are occasionally passed through the mouth (not simply between the mandibles), after which they are again applied as before."

This description is correct as far as it goes. The four cleaning reflexes, that of the antennæ, sides of the head, posterior legs and gaster, are distinctly differentiated and are so often repeated in the artificial nest that they can be readily studied under a lens of low magnification. These reflexes may be elicited with even greater frequency by powdering the ants with dry dust, chalk or plaster of Paris.

The cleaning of the antennæ, which is far and away the most frequent of these reflexes, is sometimes abbreviated, as when the ant merely raises one of her fore feet, grasps the scape or funiculus of the antenna of the same side between the strigil and the curved base of the metatarsus, passes the opposed combs along the apical portion of the appendage and again places her foot on the ground. Very often, however, the foot is carried forward directly from the antenna, thrust between the partially opened mandibles and then drawn back across the teeth, along the lower surface of the mandible and between the maxilla and labium. The maxilla is furnished with a comb very much like that on the strigil.[1]

This motion, of course, removes much of the dirt that may have been collected from the surface of the antenna, since the strigil and the fore tarsus are drawn through the clypeal macrochætæ, across the mandibular teeth, along the mandibular macrochætæ and over the maxillary comb. The function of the gular macrochætæ is not, at first sight, so apparent. Occasionally, however, I have seen an ant, while thrusting her fore foot forward, enclose between the strigil and metatarsus one or more of the long gular hairs, and draw them through the combs or along the notch between the insertions of the strigil and metatarsus. This observation, coupled with the fact that these hairs are very long, slender and directed forward, makes it highly probable that they are used for cleaning the strigil, much as we would use threads in cleaning a comb. In ants like the Old World species of *Myrmecocystus* (Figs. 8 and 9) which possess long mental but very poorly developed gular macrochætæ, the former probably answer the same purpose. The advantage to xerophilous ants of possessing a number of macrochætal brushes in addition to the strigils is obvious when we stop to consider that these insects live in dry soil, which, during long seasons of the year, is in a very friable and dusty condition. Both while traversing the surface in search of food and while carrying on their excavations, these ants are liable to become coated with dust or sand. Under such conditions the strigil must often become clogged with earthy particles or sand grains, and an apparatus for cleaning it, such as the gular and mental macrochætæ, and hairs like those on the clypeus and mandibles for brushing the fore tarsi, must be of considerable utility. Ants living in sandy deserts often have the gular hairs unusually long, and well developed, probably because sand particles, with their sharp edges, are more injurious to the delicate combs of the strigil and metatarsus and interfere more with their normal movements of occlusion than do particles of soil. If, as I believe, the circumoral macrochætæ have the function here assigned to them, they may be designated as *ammochætæ* to distinguish them from the long hairs or bristles on other portions of the ant's body.

---

[1] See Janet's figures of the mouthparts of *Myrmica rubra* in his paper entitled: "Observations sur les Fourmis," Limoges, 1904, pp. 18 and 19.

The absence of ammochætæ in several desert ants, such as the species of *Monomorium* and *Pheidole*, is probably to be explained by the very small size of the workers in both of these genera, and the fact that the soldiers of *Pheidole* do not excavate and rarely leave the galleries of the nest. For the same reason the ammochætæ are usually absent or feebly developed in the males of xerophilous species. That the workers and females of several other desert ants belonging to the genera *Solenopsis*, *Cremastogaster*, *Camponotus*, etc., should lack these structures is no more surprising than that many desert plants have failed to acquire the peculiar adaptive characters of the Cactaceæ. We may now consider briefly the occurrence of the ammochætæ in the various genera of xerophilous ants.

SUBFAMILY MYRMICINÆ.

Pogonomyrmex Mayr. — This genus embraces a number of species peculiar to North and South America and assignable to three subgenera, *Janetia* Forel, *Ephebomyrmex* Wheeler and *Pogonomyrmex* s. str. The single known species of *Janetia* (*J. mayri* Forel) occurs in Colombia. *Ephebomyrmex* comprises some four species, *E. nägelii* Forel of Brazil, *schmitti* Forel of Haiti, *imberbiculus* Wheeler of Texas and *townsendi* sp. nov. of Chihuahua. None of the species of these two subgenera has ammochætæ, and it should be noted that *J. mayri* and the first two species of *Ephebomyrmex* occur in comparatively humid regions, and that *E. imberbiculus* and *townsendi* though xerophiious, are not true desert ants. *Pogonomyrmex* s. str. comprises some twenty species, spread over portions of the high arid plains and deserts of two continents, from Montana to Patagonia, with a possible interruption in tropical Central and South America. One of the species, *P. badius* Fabr., is found in the dry, sandy regions of Georgia and Florida. All of the members of this subgenus have very well developed clypeal, mandibular and gular ammochætæ, as shown in Fig. 1, representing the head of the Mexican and Texan *P. barbatus* F. Smith, the type of the subgenus. In certain species, like *P. californicus* Buckley, which is almost exclusively confined to sandy spots in the deserts of the southwest, the gular hairs are even longer and more prominent. The species of *Pogonomyrmex* s. str. and *Ephebomyrmex* are harvesters and subsist very largely on stored seeds. According to Forel *Janetia mayri* is entomophagous. There is reason to suppose that the genus *Pogonomyrmex* represents a granivorous American offshoot of the subboreal genus *Myrmica* or of some similar but now extinct group.

Ocymyrmex Emery. — This genus was erected by Emery for four species (*barbiger*, *nitidulus*, *robecchi* and *weitzekeri*) which he described from Somaliland, Basutoland and the Cape of Good Hope. It is probable that all of these species, which resemble *Pogonomyrmex*, live in the dry plains and feed on seeds. I have examined a few workers of *O. barbiger* and *weitzekeri* and find that they agree in having well-developed clypeal, mandibular and gular ammochætæ, and also a pair of long hairs on the mentum. These hairs, as shown in Fig. 3, extend backward, diverging from their insertions, and have their tips abruptly bent forward. The gular hairs are alternately long and short, and

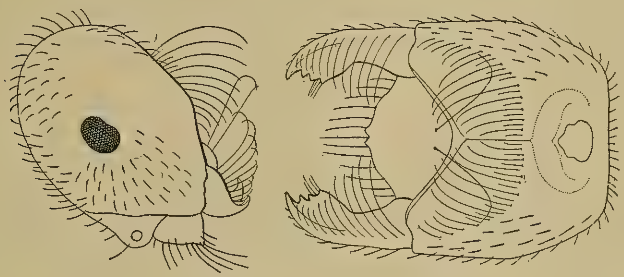


FIG. 3. *Ocymyrmex weitzekeri* Em.

the longer ones are less reclinate and shorter than in *Pogonomyrmex.* They are slightly curved upward at their tips. The mandibular ammochætæ are unusually long and curved.

Cratomyrmex Emery. — This genus is based on a single species, *C. regalis*, which Emery described from two large female specimens (19 mm. long), taken at Benue in western Africa.[1] They resemble *Pogonomyrmex* in having long hairs on the lower surface of the head, but these hairs are said to be shorter and less regularly arranged than in *P. barbatus.*

Stenamma Mayr. — This extensive genus may be divided into six subgenera: *Stenamma* s. str., *Aphænogaster, Ischnomyrmex, Messor, Goniomma* and *Oxyopomyrmex.* The species of *Stenamma* s. str., are moisture-loving ants living in small colonies in the woods of the north temperate zone. The much more numerous species of *Aphænogaster* are widely distributed and occur in a great variety of environments, some even living in dry deserts, but none of them seems to have developed ammochætæ, although the lower surface of the head, like the body in general, is usually provided with coarse erect hairs. From species of this or some very similar group the remaining subgenera, *Ischnomyrmex, Messor, Goniomma* and *Oxyopomyrmex*, seem to have been derived. All of these comprise xerophilous or deserticolous species with ammochætæ and seed-eating propensities.

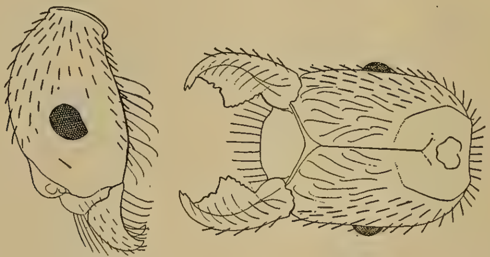


FIG. 4. *Ischnomyrmex albisetosus* Mayr.

Ischnomyrmex Mayr. — Our two North American species of this subgenus, *I. cockerelli* André and *albisetosus* Mayr are closely related and both inhabit the driest deserts of western Texas, New

---

[1] Voyage de M. Ch. Alluand dans le territoire d'Assinie (Afrique Occidentale), Formicidæ. *Ann. Soc. Ent. France*, LX., 1891, pp. 553–574, Pl. XV.

Mexico, Arizona and northern Mexico. We should therefore expect them to have well-developed ammochætæ. These are, in fact, present in both species, but, as shown in Fig. 4, the gular hairs are diffuse and not arranged in an arcuate series. They are, however, longer and more slender than the blunt, white hairs covering the remainder of the body, and are distinctly hooked or curved upward at their tips. This peculiar modification of the gular hairs is not seen in the other members of the subgenus inhabiting more humid regions, like *I. araneoides* Emery of Central America, *swammerdami* Forel of Madagascar, and *longipes* F. Smith of Burmah and Sumatra. In pilosity these resemble our northern species of *Aphænogaster.*

Messor Forel. — This subgenus of harvesting ants is represented by a number of species, subspecies and varieties in Africa, especially in the Sahara, in southern and central Europe and Asia and by five species in Arizona, Nevada, California and northwestern Mexico. In both hemispheres the xerophilous species have well-developed ammochætæ, whereas these structures are small or lacking in the moisture-loving forms. To the latter group belongs the widely distributed *M. barbarus* L. which even in Africa, according to Forel "vit dans les lieux moins secs ; fait souvent des nids maçonnés dans la terre, dans les prairies,"[1] and Lameere[2] says : "Je n'ai rencontré le type de cette moissonneuse que dans les parties cultivées des oasis ; dans le désert cail-

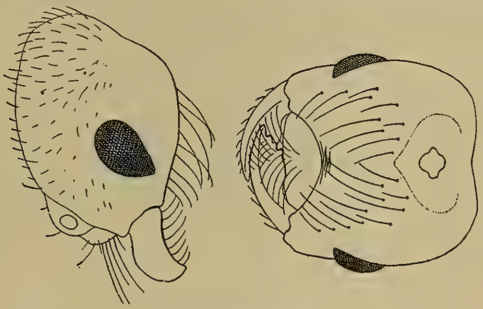


FIG. 5. *Goniomma blanci*, v. *tuneticum* Forel.

---

[1] See Emery, "Revision Critique des Fourmis de la Tunisie, in Exploration Scientifique de la Tunisie," Paris, 1891, p. 10.

[2] "Note sur les Moeurs des Fourmis du Sahara," *Ann. Soc. Ent. Belg.*, XLVI., 1903, pp. 160–169.

louteux, alluvial, j'ai toujours trouvé la race *ægyptiacus* Emery, et dans le désert rocailleux la race *striaticeps* André." Now the race or subspecies *ægyptiacus* has well-developed ammochætæ, and in *striaticeps* these bristles are also present, though more feebly developed. *M. arenarius* Fabr., and *caviceps* Forel, two species peculiar to the dry sandy portions of the Sahara, and apparently also *M. bugnioni* Forel of the same region, have highly developed ammochætæ, but these are absent in the European *M. structor*, and in *lobicornis*, a form discovered by Forel in the oasis at Biskra.[1]

Among our American species, *M. pergandei* Mayr and *julianus* Pergande have well-developed ammochætæ, whereas *andrei* Mayr, *carbonarius* Pergande and *stoddardi* Emery have only the usual hairs on the lower surface of the head. The habits of *carbonarius*, *julianus* and *stoddardi* are unknown but *pergandei*, as I can assert from my own observations, lives only in the driest portions of the deserts of Arizona and California, and *andrei* and *stoddardi* are cited only from more humid portions of the latter state. The head of *pergandei*, which is represented in Fig. 6, shows a fine development of the clypeal, mandibular and gular bristles. The gular series form an arc on each side bounding a distinctly concave median region separated from the convex lower portions of the cheek by a distinct ridge (represented in the figure by a dotted line along the insertions of the bristles).

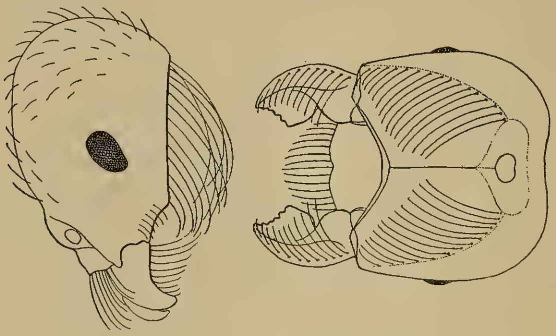


FIG. 6. *Messor pergandei* Mayr.

---

[1] "Les Formicides de la Province d'Oran (Algerie)," *Bull. Soc. Vaud. Sci. Nat.* XXX., no. 114, 1894, pp. 31–33.

Goniomma Ern. André. — Of the few forms that have been assigned to this Mediterranean genus, I have examined only *G. blanci* André var. *tuneticum* Forel from the Sahara. The head of the worker, represented in Fig. 5, shows well-developed clypeal and mandibular ammochætæ. On the gula, however, which has no distinct median suture, the bristles are diffuse and only moderately developed, and not arranged in an arc. This ant is probably granivorous like *G. hispanicum*, whose habits have been studied by Forel.

Oxyopomyrmex Ern. André. — This group, like the preceding, comprises a few small Mediterranean species. I have been able to examine a number of workers and females of *O. santschii* Forel kindly sent me by Dr. F. Santschi of Kairouan, Tunis. The ammochætæ are similar to those of *Goniomma tuneticum*. The gular bristles are diffuse and rather short, though conspicuously longer than the hairs on other parts of the body. Santschi has recently shown that this ant garners seeds.[1]

Both *Goniomma* and *Oxyopomyrmex* seem to represent depauperate offshoots of the grain-storing portion of the genus *Stenamma* (*Messor*). The depauperate character is apparent in the small size of the insects and their colonies and in the vestigial condition of the gular ammochætæ.

Holcomyrmex Mayr. — This genus of harvesting ants, apparently confined to southern Asia and northern Africa, was at first regarded by Emery[2] as being hardly distinct from *Stenamma*, but somewhat later[3] he says: " Dans ma clef analytique des genres des formicides j'ai exprimé des doutes sur la validité des charactères qui séparent le genre *Holcomyrmex* de *Stenamma*. Toutefois l'aiguillon est bien développé chez *Holcomyrmex*, faible ou rudimentaire chez tous les sous-genres de

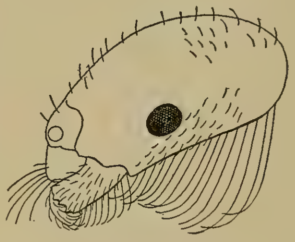


FIG. 7. *Holcomyrmex chobauti* Emery.

---

[1] See Forel, " Miscellanea Myrmécologiques, *Rev. Suisse Zool.*, T. XII., 1904.

[2] Clef Analytique des Genres de la Famille des Formicides, pour la Determination des Neutres," *Ann. Soc. Ent. Belg.*, T. XL., 1896, p. 185, *nota*.

[3] " Description d'une Fourmi Nouvelle d'Algérie," *Bull. Soc. Ent. France*, 1896, p. 419.

Stenamma. Ce charactère qui m' avait échappé à son importance me porte à croire que les analogies frappantes entre les deux genres sont dues à une adaptation convergente. *Holcomyrmex* est la modification granivore de *Monomorium*, comme *Messor* est celle de *Stenamma* (*Aphænogaster*)." There are no ammochætæ in the Asiatic species of *Holcomyrmex* (*criniceps* Mayr and *scabriceps* Mayr of India), though the lower surface of the head is abundantly pilose like the remainder of the body. These species evidently inhabit rather humid regions. In the species from the dry Sahara (*H. chobauti* Emery and *faf* Forel) the ammochætæ are beautifully developed. In *chobauti* (Fig. 7) the gular bristles are very long and inserted in an arcuate series, and the head is very flat, with concave gular surface. Forel describes *H. faf* as having long red hairs on the lower surface of the head. In *H. lameerei* Forel, which also inhabits the Sahara, the gular hairs are shorter, straighter and diffuse, but nevertheless abundant.

SUBFAMILY CAMPONOTINÆ.

Myrmecocystus Wesmæl. — This genus includes the only prominent and characteristic Camponotine ants common to the arid regions of both hemispheres. The Old World species, ranging from the plains of central Asia through central and southern Europe and north Africa to Spain and Morocco, are all very agile, entomophagous ants, which run rapidly over the dry, sunny soil in pursuit of their food. In nearly all of these

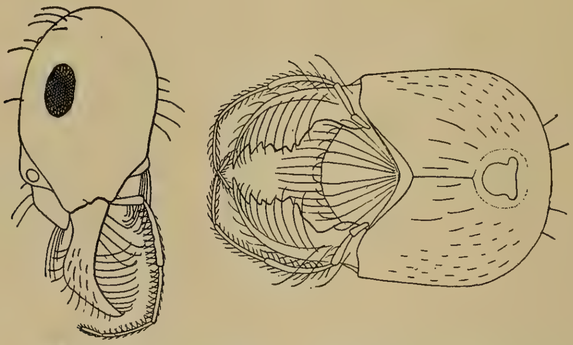


FIG. 8. *Myrmecocystus bicolor* F. (= *M. viaticus desertorum* Forel).

palearctic forms the clypeal and mandibular ammochætæ are well developed. The gular bristles, however, are vestigial, their function being usurped by a fan-shaped tuft of long recurved hairs on the mentum. It is a significant fact that the hairs of this tuft are rather short in *M. cursor* Fonsc., a species which, according to Emery does not occur in Africa.[1] It belonged originally to the central Asiatic fauna, but just after the glacial period migrated into central and southern Europe. In the north African forms (*M. albicans* Roger, *viaticus* Fabr., *bicolor* Fabr. (= *viaticus desertorum* Forel), *bicolor megalocola* Foerster) the ammochætæ, especially those on the mentum, are longer (Fig. 8). In *M. bombycinus* Roger, a typical Saharan species, the bristles reach their highest development (Fig. 9), and there are also fringes of long curved hairs on the third joint of the greatly elongated maxillary palpi so characteristic of the ants of this genus.

There seem to be only two American species of *Myrmecocystus*, *mexicanus* Wesm. and *melliger* Forel, but each of these has a number of subspecies and varieties, still in part undescribed and all confined to the arid plains and deserts of the southwestern States and northern Mexico. Some of the forms of each of the species collect the secretions of plants and the ejecta of aphids and store the liquids thus obtained in the replete workers ("honey ants"). Other subspecies and varieties do not seem to have this

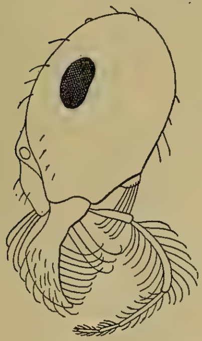


FIG. 9. *Myrmecocystus bombycinus* Roger.

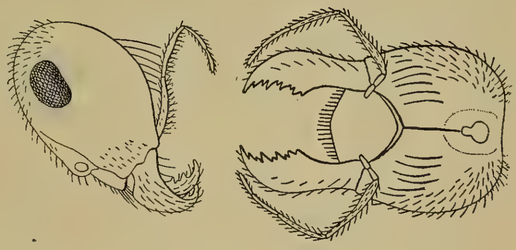


FIG. 10. *Myrmecocystus hortideorum* McCook.

---

[1] "Rassegna Critica delle Specie Paleartiche del Genere Myrmecocystus," *Mem. R. Accad. Sci. Ist. Bologna*, 1906, pp. 1–17, 35 figs.

habit but live on insect food. The clypeal and mandibular ammochætæ are smaller than in the palearctic species. There are no hairs on the mentum, but the gular bristles are long, arcuately inserted, rather stiff, directed downward and but little curved. The development of these, as well as that of the clypeal and mandibular ammochætæ varies directly as the aridity of the region inhabited by the ants. Thus *M. mexicanus* var. *hortideorum* McCook (Fig. 10) which lives on the high plains in comparatively humid regions, has very poorly developed clypeal and mandibular bristles and the gular ammochætæ are rather short. In *M. melliger orbiceps* subsp. nov. (Fig. 11) which inhabits the drier regions of central and western Texas, all of the bristles are longer. Finally *M. melliger semirufus* Emery (Fig. 12), a small form peculiar to the sandy spots in the dry deserts of Arizona and southern California, surpasses all the other American forms in the development of the ammochætæ.

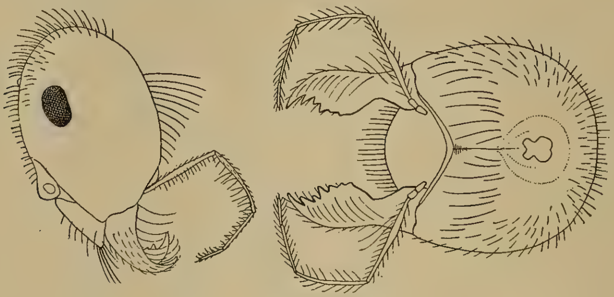


FIG. 11. *Myrmecocystus melliger orbiceps* sp. nov.

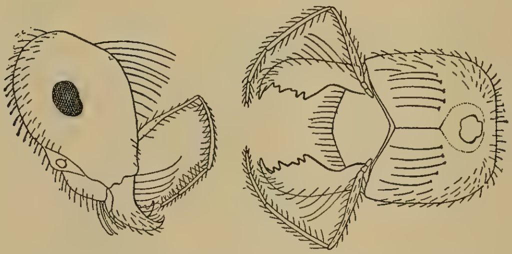


FIG. 12. *Myrmecocystus melliger semirufus* Emery.

The uniform presence of mental ammochætæ and the larger size of the males in the Old World forms of *Myrmecocystus* would seem to indicate that these are at least subgenerically distinct from the American forms. It may be advisable, therefore, to reinstate for the palearctic members of the genus the name *Cataglyphis* published by Foerster in 1850. The name *Myrmecocystus*, established in 1838 by Wesmael for *mexicanus*, would then comprise the American species.

Melophorus Lubbock. — This interesting genus embraces three subgenera: *Melophorus* s. str., *Prolasius* Forel and *Lasiophanes* Emery, all peculiar to the southern hemisphere. Many of the species of *Melophorus* proper resemble *Myrmecocystus*. In *M. bagoti* Lubbock and *M. wheeleri* Forel, which inhabit the most arid portions of Australia and have dimorphic workers (*M. bagoti*, at least, being a honey ant, as Lubbock has shown !), the ammochætæ are highly developed and all the series are present, even to the tuft on the mentum. The gular series are longer and more abundant than in the American species of *Myrmecocystus*. These hairs are less developed in *M. iridescens* Emery, *æneovirens* Lowne, *curtus* Forel, *hirtus* Forel, and *ludius* Forel, as I have found from an examination of specimens of all of these forms in Professor Forel's collection. In *M. nitidissimus* André and *formicoides* Forel all the series of ammochætæ are inconspicuous, so that these forms may be taken to represent a transition to the *Lasiophanes* species of Chile and *Prolasius advena* F. Smith of New Zealand, which are not deserticolous and have no prominent hairs on the lower surface of the head and mandibles.

SUBFAMILY DOLICHODERINÆ.

Dorymyrmex Roger. — This peculiarly American genus is the only one of the Dolichoderine subfamily in which I have found ammochætæ. A single species, *D. pyramicus* Roger, with at least three varieties (*niger* Pergande, *flavus* McCook and *bicolor* Wheeler) is widely distributed through the subtropical and tropical portions of both continents. It lives exclusively in dry soil or sand, preferably in the latter. More numerous species occur in the dry regions of Argentina, Patagonia and Chile, which probably represent the original home of the genus. Several of these species (*D. planidens* Mayr, *mucronatus* Emery, *tener* Mayr and *baeri* André) have well-developed ammochætæ especially on the clypeus and gula. I find that the conditions shown in Fig. 13, taken from one of Emery's recent papers,[1] obtain in all of these species. The gular macrochætæ are arranged in an arc on each side and the insertion of each bristle is marked by a black dot. In our North American forms of *D. pyramicus* (Fig. 14), the ammochætæ are reduced to a very prominent series on the clypeus and a few short bristles, inserted in a short irregular arc on the middle of the gula. The latter, which are all the more conspicuous because the remainder of the body is nearly destitute of hairs, nevertheless represent what may be regarded as a degenerate condition.

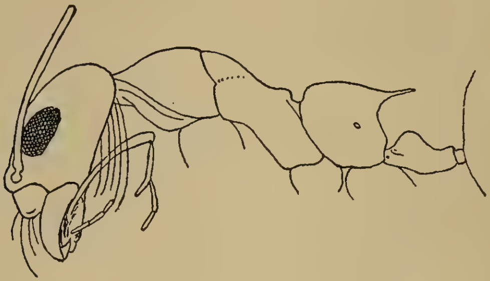


FIG. 13. *Dorymyrmex mucronatus* Emery.

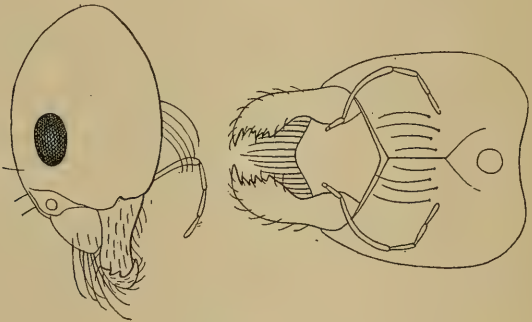


FIG. 14. *Dorymyrmex pyramicus* Roger.

---

[1] "Studi sulle Formiche della Fauna Neotropica," *Bull. Soc. Ent. Ital.*, XXXVII., 1905, Fig. 34.

CAN SEA WATER MAINTAIN THE BEAT OF THE HEART OF FRESH WATER ANIMALS?

THEO. C. BURNETT.

(From the Rudolph Sprecles Physiological Laboratory of the University of California.)

In 1900 Loeb[1] introduced the idea of physiologically balanced solutions, *i. e.*, "salt solutions which contain such ions in such proportions as to annihilate completely the poisonous effect which each constituent would have if it were alone in solution." On this basis blood, sea water and Ringer's solution are examples of balanced solutions. Loeb[2] was led to this idea by the observation that young *Fundulus*, which live in sea water, can also live in distilled water, while they die rapidly in a pure solution of sodium chloride (or any other salt) of the same osmotic pressure as sea water. Now we know that the life of the heart is sustained in blood and in Ringer's solution; the question arises is it equally well sustained in sea water that has been made isotonic with the blood. We have already a few facts in this regard. Rogers[3] found normal sea water to be an excellent sustaining fluid for the heart of the marine crab, and Garrey,[4] says "the mammalian muscle lives longer in isotonic sea water than in any other inorganic solution tested." Osterhout[5] experimenting with *Vaucheria sessilis*, a green alga common in running water, found it would grow well and live indefinitely in sea water with a concentration approximating 3/32 *M.* According to Van't Hoff the composition of sea water is as follows: 100 mols. NaCl; 2.2

---

[1] Loeb, J., "On the Artificial Production of Normal Larvæ from the Unfertillized Eggs of the Sea Urchin (Arbacia)," *Am. Jour. Phys.*, Vol. 3, 1900, p. 434. Also "Studies in General Physiology," p. 590.

[2] Loeb, J., "On Ion-proteid Compounds and Their Rôle in the Mechanics of Life Phenomena," *Am. Jour. Phys.*, Vol. 3, 1900, p. 327.

[3] Rogers, C. G., "The Effects of Various Salts upon the Invertebrate Heart," *Jour. Exper. Zoöl.*, Vol. 2, 1905, p. 237.

[4] Garrey, Walter E., "Twitchings of Skeletal Muscles Produced by Salt Solutions, etc.," *Am. Jour. Phys.*, Vol. 13, 1905, p. 186.

[5] Osterhout, W. J. V., "Extreme Toxicity of Sodium Chloride and its Prevention by Other Salts," *Jour. Biol. Chem.*, Vol. 1, 1905–6, p. 363.

mols. KCl; 2 mols. $CaCl_2$; 7.8 mols. $MgCl_2$; 3.8 mols. $MgSO_4$; reaction alkaline. Sea water thus differs from Ringer's solution in containing magnesium, while it differs from blood only in the excessive amount of magnesium present. This being the case it was determined to try its effect on the heart of the fresh water turtle.

The technique employed was essentially that used by physiologists in experiments of this nature. Strips of the ventricle were prepared in the usual way and attached by means of platinum hooks to a light aluminium lever magnifying about nine times. In order to have controls the strip was sometimes divided longitudinally, and sometimes transversely, as Martin[1] has shown there is no difference in the action of the strips (except the amplitude), whether prepared one way or the other. By dividing transversely strips are obtained containing fibers of both the anterior and posterior wall of the ventricle, which appeals to one as being advantageous for comparison. The lever was not weighted except when the whole heart was used, in which case a one gram weight was added just far enough from the fulcrum to about balance the weight of the heart. The lower end of the strip was attached to a glass rod bent at right angles, which served as a fixed point. Cylinders containing about thirty-five cubic centimeters of solution were used to immerse the strips. In the case of the sinus and auricles, they were separated completely from the ventricle by an incision in the auriculo-ventricular groove, and suspended in the same way as were the ventricular strips. The sea water used was taken from the Pacific Ocean at a point about a mile below the Cliff House, San Francisco. It had a freezing depression of 1.85. It was made isotonic by dilution with water distilled in glass, and its concentration determined from time to time by the freezing point. Twenty-eight c.c. of sea water in 100 gave the same depression as the Ringer's solution used for controls, which was made up according to the following formula: 100 mols. NaCl; 2 mols. KCl; 2 mols. $CaCl_2$; trace of $NaHCO_3$; all of $m/8$ concentration. Unless otherwise stated when "Ringer" is referred to, this solution is meant.

---

[1] Martin, E. G., "An Experimental Study of the Rhythmical Activity of Isolated Strips of Heart Muscle," *Am. Jour. Phys.*, Vol. 11, 1904, p. 103.

The turtles used were mostly those common to this locality (California), but a few were from Illinois — the *Emys Meleagris.*

It may be as well to begin by stating that the turtle's heart will remain alive as long in dilute sea water as in "Ringer." By "alive" is meant that condition of the heart in which, though quiescent it still responds to a stimulus by a contraction. Usually an induction current was used to ascertain if the heart would still contract. Individual hearts vary within such wide limits that in experiments on the whole heart controls were considered of such doubtful value that they were seldom used. The temperature also is a factor, for in warm weather bacterial decomposition soon sets in. In cool weather records of the ventricle have been obtained extending over one hnndred hours; but most of this work was done at higher temperatures and the hearts would not last as long either in sea water or in "Ringer." The auricles have given an uninterrupted series of contractions lasting eighteen hours or more, in cool weather.

When a strip of ventricle from a turtle's heart is immersed in isotonic sea water, it remains perfectly quiet indefinitely. Occasionally it will give a single spontaneous contraction, but it has been known to remain for twenty-four hours without giving a single beat. The shortest time in any of my experiments that it remained quiet was three hours and three quarters, when it gave one contraction, followed by two more eighteen hours later. Control strips in "Ringer" would invariably give single contractions or groups of contractions, separated by periods of quiescence, and were always more active than the corresponding strips in sea water. If, however, the strips were first treated with pure NaCl in $m/8$ concentration until "sodium chloride arrest" was induced, the difference was more marked. The strip transferred to "Ringer" usually recovered and gave a good series of contractions; while that transferred to sea water would revive for a few minutes and then become quiet, giving contractions of a very slow rate, or even none at all. That it was still capable of contraction was demonstrated by its response to stimulation with the induced current.

In striking contrast to the apical strips was the behavior of the auricles, always taken from the same heart for comparison. As is well known when they are suspended the auricles contract spontaneously. When placed in dilute sea water the rate was always increased by several beats per minute, and they gave an uninterrupted series of contractions gradually diminishing in rate and amplitude, lasting from seven to twenty-eight hours. Usually when longer than twelve to eighteen hours, towards the last the contractions would be irregular, with pauses between groups. Control auricles in "Ringer" showed practically no difference. They too exhibited a slight increase in rate and gave the same characteristic series of contractions — the average being about fourteen hours in both "Ringer" and sea water, with the longest and the shortest records to the credit of the sea water. The following description of a few experiments will supply some details.

Exp. Jan. 28/'07; temperature 17° C. Strip *A* was placed in Ringer's solution. During the first four hours no contractions occurred. During the next four hours there were four series of rapid contractions, each series lasting from two to five minutes, with one half to one hour between. In ten hours from beginning the experiment the strip failed to respond to the induced current. Strip *B* was placed in dilute sea water. During ten hours it did not give a single contraction. At the end of this time it still responded feebly to the induced current. The auricle of this heart was suspended in dilute sea water and gave a fine series of contractions lasting about three hours. Before immersion the rate was sixteen per minute; after immersion it was eighteen, becoming slower toward the end of the three hours. This was the shortest series of contractions obtained. For some unaccountable reason both the ventricle and auricles gave out in a much shorter time than usual.

Exp. Feb. 1/'07; temperature 20° C. Two strips of the ventricle were prepared in the usual way. Strip *A* was put in Ringer's solution, and after a latent period of twenty-five minutes, gave a single vigorous contraction. It then remained quiet for twenty minutes, when it again began to contract, and gave a series of contractions lasting about ten hours, the rate varying from one to eight per minute. Twenty-two hours after beginning the experiment it was stimulated with the induced current, but gave no response. Strip *B* in dilute sea water remained perfectly quiet for eleven hours. It was then stimulated with the induced current, and gave five vigorous contractions of maximum amplitude after which it was again quiescent. Twenty-two hours after beginning the experiment, it was again stimulated and gave a fairly good contraction of about one third the maximum in height. The solution was turbid indicating bacteria, and two hours later it failed to respond to stimulation. The auricle of this heart was placed in "Ringer" and gave a fine series of contractions lasting eleven and one half hours, with an initial rate of twenty, which was increased to twenty-four after immersion in "Ringer."

Exp. Feb. 4/'07; temperature 18° C. Two strips of the turtle's heart were prepared as usual. Both strips were put in *m*/8 NaCl in order to bring them to "sodium chloride arrest." Strip *A* began contracting after a latent period of fifteen minutes; its rate, five per minute, increasing to fifteen with gradually diminishing amplitude. At the end of two hours and fifteen minutes it ceased contracting. After remaining quiet for fifteen minutes it was transferred to "Ringer." It began at once to recover, but the contractions were small in amplitude, accompanied by a strong rise of tone. The rate was nine, increasing to fifteen per minute. Nine and one half hours from beginning the experiment, it responded to electrical stimulation by a very feeble twitch. Strip *B* began contracting after a latent period of fifteen minutes. Its rate was four per minute increasing to twelve. It came to "sodium chloride arrest" at the same time as did strip *A*, and was transferred to dilute sea water. It began contracting at once with a rate of nine per minute. After about fifteen minutes it ceased suddenly for five minutes and then began centracting feebly and in groups. It then settled down to a slow rate of one or two per minute, but with increased amplitude, and continued this for four and one half hours. It was stimulated with the induced current at the same time as was strip *A*, and gave a single contraction that was more vigorous than strip *A*, although it was still feeble. The auricle was contracting in the air at a rate of twenty per minute. After it was put in sea water the rate was twenty-four. It gave an uninterrupted series of contractions lasting about nine hours. After a long rest it recovered a little and gave some weak contractions. The duration of its irritability was about nineteen hours, nine of which were spent in vigorous activity.

These three experiments were selected because they came in a series, and it so happens that it includes two of those that are the least typical of any. Exp. Jan. 28/'07 as has been said, lived for a much shorter time than usual, while in exp. Feb. 4/'07 the turtle was blind and sluggish ; when the plastron was removed the heart was not contracting, nor did the auricles contract until they were suspended.

Exp. Feb. 8/'07 ; temperature 17–21° C. Two strips of the turtle's ventricle were prepared and exhausted in NaCl. Strip *A* was then transferred to dilute sea water. At the end of six minutes it began contracting vigorously with a much greater amplitude than in NaCl ; at first with a rate of six per minute, but soon dropping to one in two or three minutes. In about fifteen minutes it ceased abruptly and remained perfectly quiet for about three quarters of an hour. It was then transferred to "Ringer," and in seven minutes began giving maximum contractions with a somewhat irregular rhythm ; there would be a group of six to eight per minute, then a pause for half a minute and then another group. When it had been contracting in this way for one and one half hours it was again transferred to sea water. It was then registering on a twelve hour drum and the rate was not taken ; but in fifteen minutes it again ceased abruptly and remained perfectly quiet for between five and six hours. Again transferred to "Ringer," it remained quiet for half an hour and then began to contract vigorously, but with gradually diminishing amplitude, and during the night it ceased. Next morning it failed to respond to stimulation. Strip *B* was used as a control. After NaCl exhaustion it was placed in "Ringer" instead of sea water, and recovered at once with a rate of sixteen gradually dropping to twelve per minute. The subsequent course of this strip was similar to strip *A* ; when *A* was in "Ringer," *B* was in sea water and *vice versa*. When *A* was active in "Ringer," *B* was quiescent in sea water. The record of the auricles was lost after seven hours.

Having ascertained the influence of dilute sea water upon the different parts of the heart separately, the whole heart was suspended by passing a hook through the apex below and one through the auricle above. In this way one can obtain a record of both the ventricular and the auricular contractions in their relations one to the other. The results of several experiments show that sea water favors the relaxation of the ventricle, while it has either no effect or a slightly stimulating one upon the auricles. The tracings show a more orderly sequence of auricular systole followed by ventricular systole, than in "Ringer," where the auricular systole has a tendency to fuse with the ventricular, owing possibly to incomplete relaxation of the ventricle. The question is being studied further in connection with the effects of magnesium on the heart; and as this paper is in the nature of a preliminary communication, the discussion of it will be left to a future date.

As the only practical difference between sea water and "Ringer" is the magnesium present in the former, it seems more than likely that the latter salt is responsible for the difference in the effect of these two solutions. As in the case of the marine crab, so in the turtle's heart, sea water rendered isotonic with the blood seems to be an excellent sustaining fluid; in other words sea water isotonic with the blood is a "physiologically balanced solution" for the turtle's heart. It is conceded that sea water contains magnesium in excess of that usually found in blood, and it is for that very reason it has proved useful in pointing the way for further research; but while it is found in the blood in the proportion of about two of magnesium to three or more of calcium (Hammarsten), the conditions are reversed in muscle and nerve, the proportion being about two of magnesium to one of calcium. One would therefore expect the quantity of magnesium to vary considerably at different times, and it is difficult to say what constitutes an excessive amount; an optimum has yet to be determined.

In conclusion it should be said that these results were to be expected in the light of those of Quinton,[1] who not only transfused dogs with isotonic sea water in quantities equal to the quantity of blood withdrawn, but also injected large quantities intravenously, without serious consequences. "Entre l'eau de mer et le milieu vital du Vértebré, il y a physiologiquement identité."

---

[1] Quinton, R., "L'Eau de Mer Milieu Organique," 1904, p. 160.

SUMMARY.

1. Strips of the ventricle of the turtle's heart will live as long in isotonic sea water as in Ringer's solution.

2. After "sodium chloride arrest," strips of ventricle will recover as well in isotonic sea water as in Ringer's solution.

3. The whole heart will beat as long in isotonic sea water as in Ringer's solution.

This problem was suggested to me by Professor Loeb, and I have to thank him for many helpful hints.

NOTE. — At the time these experiments were begun and the paper was in preparation, I was ignorant of the work of Mayer[1] on the embryo heart of the loggerhead turtle, which should be referred to.

---

[1] Mayer, Alfred G., "Rhythmical Pulsations in Scyphomedusæ," Carnegie Institution of Washington, Publication 47, 1906.

ON THE RELATIONSHIP OF THE FISHES OF THE FAMILY SIGANIDÆ.

EDWIN CHAPIN STARKS.

Stanford University, California.

The relationship of the family Siganidæ has long been uncertain, though modern authors are unanimous in placing it near the family Acanthuridæ, which it resembles in form of body and in other external features. It was with the hope of finding some skeletal characters that might further indicate its relationship that this investigation was undertaken. The form chosen to represent its family was *Siganus fuscescens* from Japan.

Descriptive.

The cranium does not depart very much in shape, nor with few exceptions the elements composing it very much in shape, size, or arrangement, from the percoid cranium with the superior ridges little developed as in the genus *Perca*. The myodome is well developed and opens widely to the exterior at the posterior end of the parasphenoid. The basisphenoid is absent, but at the front of the myodome in the usual place of the descending process from the basisphenoid the interorbital tissue is thickened and separates the eye muscles exactly as the basisphenoid process usually does. The alisphenoids are remote from each other. The exoccipitals meet on the superior surface of the basioccipital; the vagus foramen in the exoccipital is very large. The crest of the superoccipital is moderately developed, but does not reach to the anterior end of that bone. The frontals are large spongy bones, evenly convex transversely, and without ridges or canals. Posteriorly they reach to the epiotics at each side of the supraoccipital which is wedged in between them. To their anterior ends are attached the wide thin nasals, which are joined to each other at the median line and appear like a continuation of the frontals, arching widely over the nasal chambers forming a rounded roof open only outward and downward. The ethmoid is ossified some distance in front of the prefrontals, and is interposed between the vomer and the nasal bones. Posteriorly it is continued backwards as a cartilaginous plate separating the prefrontals and behind them is connected with the tissue of the interorbital septum. The prefrontal is not pierced by the olfactory nerve, but the nerve runs to the nasal chamber through a passage

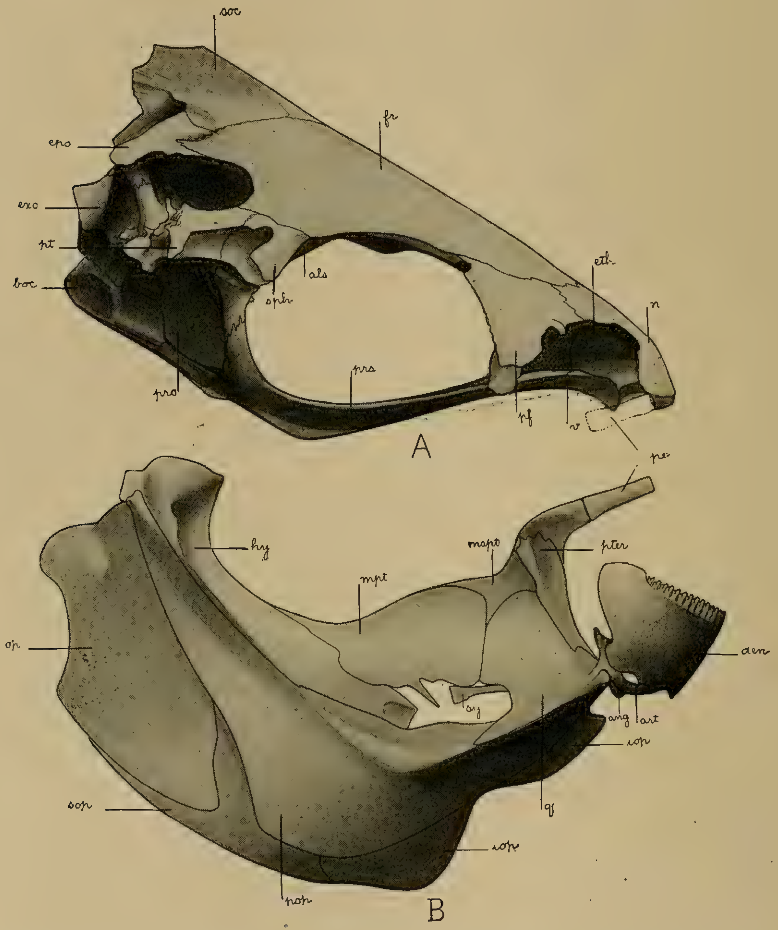

soc
fr
epo
exo
pt
boc
als
sph
eth
n
prs
pro
pf
v
pe
A
hy
mspt
pter
mpt
op
den
sy
ang
art
iop
sop
q
iop
pop
B

FIG. I. *Siganus fuscescens.* *A*, lateral view of cranium; *B*, lateral bones o head. *als*, alisphenoid; *ang*, angular; *art*, articular; *boc*, basioccipital; *den*, dentary; *epo*, epiotic; *eth*, ethmoid; *exo*, exoccipital; *fr*, frontal; *hy*, hyomandibular; *iop*, interopercle; *n*, nasal; *mpt*, metapterygoid; *mspt*, mesopterygoid; *op*, opercle; *p*, palatine; *pe*, prepalatine; *pf*, prefrontal; *pop*, preopercle; *pro*, proötic; *prs*, parasphenoid; *pt*, pterotic; *pter*, pterygoid; *q*, quadrate. ; *soc*, supraoccipital; *sop*, subopercle; *sph*, sphenotic; *sy*, symplectic; *v*, vomer.

between the prefrontal and the ethmoid. The vomer is branched or Y-shaped and toothless. The opisthotic is present in its usual position covering the suture between the exoccipital and the pterotic. The parietal is entirely absent, and the frontal and epiotic bound the supraoccipital.[1] The remaining cranial elements are typical of the majority of the spiny-rayed fishes and are well shown in the accompanying drawings.

The post-temporal is forked and though the lower fork is very short it is continued by a ligament and joined to the opisthotic in the usual way so that the post-temporal stands away from the cranium as it does when the lower fork is long. The superior fork is rather broadly but not firmly joined to the epiotic. A couple of tunneled dermal bones, the supratemporals, are present on each side of the cranium in front of the post-temporal. The hypercoracoid foramen is large and is directly in the center of the bone. The actinosts are moderately long and somewhat constricted in the middle; three of them join the hypercoracoid and one the hypocoracoid. The upper pectoral ray works directly on the edge of the hypercoracoid. The postclavicle is a long, slender, curved ray of bone composed of an upper and a lower element. The other shoulder girdle elements are typically percoid in size, shape, and arrangement.

The lower part of the pelvic girdle extends forward as a long roughened plate just under the skin of the breast. From the upper surface of this plate the girdle is developed vertically upward, and meeting its opposite fellow at the upper edge, which is inclined towards it, incloses a chamber between. Anteriorly a long spine is sent forward from the upper edge of the girdle to between the clavicles. Backward over the base of the ventral fin a triangular spine is developed. The posterior or inner ventral fin spine is attached to the lower surface of this triangular pelvic spine — the anterior or outer ventral fin spine is attached to the base or posterior end of the breast plate. Some space is left between the ventral spines in which the three ventral rays are placed with their bases close together nearer the posterior ventral spine than the anterior.

[1] Several crania of different sizes (the smallest from a specimen 7 cm. in length) were prepared and examined on both the inner and outer surfaces, but no trace of the parietal was found.

The maxillary elements are of rather thin spongy bone rather solidly and immovably attached to each other though not anchylosed. The premaxillary carries a single row of bicuspid teeth (or tricuspid teeth if a scarcely developed third cusp be considered). The usual backward developed spine from the symphysis of the premaxillaries is scarcely developed, and instead of sliding over the vomer and ethmoid between the nasals, it abuts against the shallowly concave front of the ethmoid. A short process from each maxillary fits into a cup at the front of each arm of the vomer, and the movement of the upper jaw is a swinging motion from a hinge similar to that of the mandible rather than the usual sliding motion.

The opercular apparatus is complete and in no way peculiar. The preopercle has an extra long lower limb extending forward from its angle with the upper limb. The hyomandibular is long and with a simple unforked head. It sends no process to the metapterygoid, which occupies a position between the lower end of the hyomandibular and the quadrate. The mesopterygoid is very small but in the usual position between the metapterygoid and palatine and connected with the pterygoid below. The symplectic is small and slender and runs along the inner surface of the quadrate. The pterygoid and palatine are normal in arrangement, the latter attached to the lower edge of the prefrontal, but anterior to the palatine is a prepalatine bone. This is a cylindrical-shaped bone, suturally, but not immovably attached to the front of the palatine and extending anteriorly along the side of the vomer to the upper edge of the maxillary, very much as the anterior process on the palatine in the majority of spiny-rayed fishes does.[1]

The mandible is short and resembles the united maxillary bones in shape. The articular is very small and is almost covered from sight by the dentary. A small angular bone is present. The mandibular teeth are in a single row and similar to those on the premaxillary.

The suborbital chain is complete, but no suborbital shelf extends inward around the orbit.

---

[1] In order to make sure that the possession of this unique prepalatine element was normal three different specimens were examined.

There are two basibranchials; the hypobranchials of the fourth arch and the pharyngobranchials of the first are absent. There are three pharyngobranchials present on each side, each a thin concavo-convex plate, shaped like a clam shell, and bearing along its lower edge a single row of long, slender, comb-like teeth. A second, less complete row is situated towards the convex center of the plate, though on the first plate the second row is represented by a single tooth. Similar teeth are arranged in four or five "combs" placed obliquely across each slender lower pharyngeal. A third or more of the band of gill filaments of the first arch (and a decreasing portion of it on the succeeding arches), is free from the arch above its angle at the upper end of the ceratobranchial, and rises upward on a cartilaginous base at each side of the cranium in a cavity behind the eye.

The hyoid arch is in no way peculiar in form or arrangement of its elements, except that the paired hypohyals are larger than usual; a small glossohyal is present.

The vertebræ number as follows: thoracic $10 +$ caudal $12 +$ hypural $= 23$.

The first vertebra has a wing of thin lace-like bone developed outward and downward from the side of its neural arch to which the first epipleural is attached. The parapophyses are scarcely developed, but their small representatives are of about the same size on all of the vertebræ. The ribs are attached to the centræ of the vertebræ with the anterior edges of their bases fastened closely against the parapophyses. The epipleurals posterior to the first are attached to the ribs at some distance from the vertebræ. The spine bearing interspinous bones are somewhat wider than the ray bearing ones. They expand laterally at the bases of the spines, making a row of bony plates, which are evident through the skin of the entire fish. The hæmal, neural, interhæmal, and interneural all have a thin laminæ of bone developed backwards from their posterior edges. A long, strong process extends forward from the first interhæmal towards the pelvic girdle and forms a sharp abdominal ridge. A sharp spine projects forward from the first interneural at the base of the first dorsal spine and pierces the skin at the nape. The supplementary caudal rays are attached to the backward extending spinous processes of one or two vertebræ anterior to the hypural.

RELATIONSHIPS.

Dr. Gill in the "Standard Natural History"[1] places the family Siganidæ with the Acanthuridæ under the superfamily Teuthidoidea. The Teuthidoidea he believes to be descended from the Chætodontoid fishes while the plectognathous fishes are descended from the Teuthidoidea.

Dr. Jordan in his "Guide to the Study of Fishes"[2] follows the same arrangement but he places the families Acanthuridæ and Siganidæ together with the Chætodontidæ and other related forms in a large group, the Squamipinnes, though he considers the Acanthuridæ and the Siganidæ under different suborders, giving to the latter suborder the name Amphacanthi.

Either of these, with slight changes, is the order in which these fishes are arranged by all modern authors, and as it is apparently the most logical no other arrangement need be here referred to.

Though there is doubtless an alliance between the families Acanthuridæ and Siganidæ the alliance is certainly not close enough to place them in the same superfamily. The Teuthidoidea is defined by the "development of transverse, expanded, buckler-like, subcutaneous plates on the back intervening between the spines, and limiting their erection forward."

The expanded interspinous rays that form the bony bucklers at the base of the spines are developed in this respect only to a slightly greater degree than may be found in many scombroid fishes, and not to so great a degree as in some berycoid fishes.

The fishes of the Acanthuridæ[3] differ from those of the Siganidæ in having the cranium wedge-shaped or tapering to a point at the ethmoid region, the parasphenoid drawn out downward into a wide, thin, sharp plate before the orbital region; the post-orbital part of the cranium shortened; the parietal present; the post-temporal suturally attached to the cranium and forming an integral part of it (this true of *Hepatus* to a greater extent than of

---

[1] Cassino & Co., Boston, 1885, Vol. III.

[2] Henry Holt & Co., New York, 1905, Vol. II.

[3] *Hepatus bahianus* and *Xesurus punctatus* represent the family Acanthuridæ in this investigation.

Xesurus)[1] the parapophyses developed on all the vertebræ; the postclavical composed of a single piece, though of a similar long, slender shape to that of *Siganus*.

In spite of these differences *Siganus* appears to be more closely related to the family Acanthuridæ than to any other known group. It resembles the fishes of the family Acanthuridæ in having the maxillary and mandibular elements short and resembling each other in shape; the maximillary and premaxillary solidly attached to each other; the spines from the symphysis of the latter short; and the movement of the maxillaries similar to that of the mandible; the teeth flattened, in a single row, and without entire edges; the suborbitals without a shelf below the eye-ball; the gill-filaments extending free above the gill arches in a cavity at each side of the cranium; a spine developed forward from the first interneural spine; the gill openings restricted to the sides.

In the connection of the maxillary elements to the cranium *Siganus* resembles *Balistes* and the other plectognathous fishes, in which the maxillary elements fit against the concave front of the ethmoid region and have a hinge-like movement without any sliding motion forward. In the acanthuroid fishes the ethmoid region is hemispherical in front, and the maxillary elements fit socket-like over it, with very short premaxillary spines above. A movement similar to that in *Siganus* is produced by the maxillary elements turning on the ethmoid ball so that the premaxillary spines glide over the ethmoid somewhat as in the majority of fishes, but there is no sliding motion straight forward.

In most characters, however, the acanthuroid fishes resemble *Balistes* more closely than does *Siganus*. The shape of the cranium is strikingly similar: tapering forward; the parasphenoid extending down in a wide thin plate; the postorbital region shortened; the sphenotic and prefrontal regions curving around the eye; and the postclavicle a long simple ray of bone.

In all of the plectognathous fishes the premaxillary is firmly anchylosed to the maxillary. In the Acanthuridæ and Siganidæ these elements are immovably attached to each other but are held together only by connective tissue.

---

[1] The attachment of the post-temporal to the cranium apparently has not the importance sometimes given to it as Dr. Gill has shown in his paper on the affinities of the Ephippiids (Proc. U. S. Nat. Mus., Vol. V., p. 557), or as the present writer has shown in his work on the shoulder girdle of the Hemabranchiate fishes (Proc. U. S. Nat. Mus., Vol. XXV., p. 619).

The peculiar pelvic girdle of *Siganus* has its counterpart in the berycoid fishes, but in this connection probably means nothing. The modification of the girdle is brought about by a development upward of the middle portion so that a chamber is inclosed between the opposing sides.

SUMMARY.

Siganus stands rather widely away from any known form. The possession of the peculiar prepalatine element and the two spines to each ventral preclude a close relationship to any living fishes. Though its relationship to the acanthuroid fishes is not close it apparently was descended from some form near that stock, and the condition of the maxillary elements, particularly their attachment to the cranium, indicate a relationship in the plectognathous direction rather than in the Chætodontoid.

If this be true *Siganus* can only be an off-shoot at one side from some acanthuroid form having the plectognathous articulation of the upper jaw to the cranium. The acanthuroid fishes are in a more direct line with the plectognathous fishes and *Siganus* could not stand between them.

The plectognathous fishes show degeneration from the acanthuroid stock by a series of continuous and ever increasing steps. *Siganus*, on the contrary, shows development in the direction of higher specialization.

The characters of *Siganus* are apparently of sufficient value to entitle it to independent superfamily rank at least, or to a rank coördinate with that of the acanthuroid fishes.

THE FERTILIZATION OF AMŒBA PROTEUS.

GARY N. CALKINS.

Three years ago I published a short paper entitled "Evidences of a Sexual Cycle in the Life-history of *Amœba proteus*"[1] in which I described the formation of chromidium in one life-phase of this common rhizopod and the subsequent formation of secondary nuclei. The latter were interpreted as nuclei of the supposed gametes which were collected in a cyst. The supposed gametes were in no case seen to emerge from the cyst and conjugation was not observed. Many of the structures observed in the amœbæ at this time, could not be interpreted in terms of the corresponding phases of other fresh water rhizopods, a curious division of the granules (represented in Plate 3, Fig. 23 of the former paper), and an equally enigmatical series of spheres with peripheral granules (represented in Figs. 12, 24 and 27), being particularly hard to homologize with other known phases in rhizopod development. Had I not been busy with other work at the time, I might have discovered that the very material used for these "evidences" would furnish proof of the actual fertilization, for this last spring, hoping to find some trace of a maturation process in rhizopods at the period of chromidium formation, I dissolved off the cover glasses from the amœbæ which were preserved in balsam, embedded them in paraffine, cut them one by one in sections from three to five microns in thickness and discovered the method of fertilization. A careful examination of the so-called "dividing granules" in these sections revealed the fact that what I had interpreted in the total mounts as dividing forms of the chromidial granules, were actually minute nuclei in the process of fusion, and that, instead of division, it was the process of fertilization, while the encysted bodies with the peripheral granules were stages in the development of these fertilized nuclei. The material, unfortunately, is still not complete enough to give the details of the chromatin changes as thoroughly as I wish, but there are enough stages to enable us to clear up this sexual phase in the cycle of *Amœba proteus* and to bring together the observation of Scheel and earlier observers and to combine them in one completed life history.

---

[1] *Arch. f. Protistenk.*, V., 1904, pp. 1–16.

As described in my earlier paper, this phase of the life-history of *Amœba proteus* is characterized by the repeated division of the nucleus until many nuclei are present in the cell, 72 to 80 being the largest numbers observed in any one organism. These nuclei then fragment, and the chromatin granules, liberated by rupture of the nuclear membranes, are distributed in the cytoplasm. This fragmentation continues until all but one of these primary nuclei are thus broken up, this one remaining as a residual nucleus, while the cytoplasm becomes packed with the chromatin fragments which I had interpreted as the chromidium. Stages in this disintegration of the nuclei are shown in Figs. 6, 7, 8, 19–26, of my earlier paper. These granules were described as increasing in size, dividing and ultimately forming the hollow spheres with peripheral granules in the encysted stage (Figs. 23, 24 and 27 of the former paper).

Now that the amœbæ have been removed from the slides, sectioned, and stained in iron-hæmatoxylin, the structure of the granules is brought out with more vivid clearness than in the total preparations stained with picro-carmine. The disintegration of the primary nucleus can be followed step by step in the sections and the origin of the gametic nuclei, as I may now call them, can be easily traced.

The first indication of fragmentation is the collection of the chromatin about the inner walls of the primary nucleus. Comparatively large reservoirs are massed about the periphery in this way (Fig. 1), but in the meantime chromatin granules in the interior of the nuclei are assuming a definite form, while a less deeply staining, more homogeneous cortical zone of nuclear plasm collects around them. The peripheral granules are also used to form similar minute nuclei which, apparently as soon as formed, move out into the cytoplasm. Here they are clearly marked nuclei, consisting of a densely staining central granule or karyosome with a more faintly staining cortical zone (Figs. 2, 3, 4, 5, 6, 7). The bulk of the primary nuclei is metamorphosed into these secondary nuclei, which are so small and so numerous that they give a characteristic granular appearance to the cell.

The earliest stages of secondary nucleus formation within the nucleus are so minute that they would scarcely be taken for the same things as the cytoplasmic nuclei. Stages in growth, however, can be found in which the size varies from these extremely minute ones to the full size nuclei of the cytoplasm (Figs. 1, 2). Fig. 5 shows a primary nucleus in the process of fragmentation with two full size secondary nuclei emerging at *a* while within the nucleus one or more large ones can be made out. Figs. 6 and 7 and Fig. 8 show similar late stages in secondary nucleus formation.

After emerging from the primary nucleus the secondary or gametic nuclei fuse and the stages in the process can be followed step by step in the fixed material. In such fixed material, however, the argument may be raised that it is equally possible to trace the history of stages in the opposite direction and claim that the process is one of division and not conjugation, this being one of the serious difficulties in working on preserved organisms. Nevertheless the evidence is so strong that there exists no doubt whatsoever in my own mind that we are dealing with conjugation and not with division. In the first place the number of gametic nuclei is far greater than the number of sporoblasts which make up the later cyst stage. In one specimen I counted more than three hundred gametic nuclei in addition to eighteen as yet unfragmented primary nuclei, while the number of sporoblasts in the later stages does not exceed 250 in any one of the specimens in my possession. Numerical relations indicate, therefore, that union rather than division takes place. In the second place the individuals of the pairs of nuclei that are fusing, are of the same size as the single ones. If they were dividing the daughter nuclei would be considerably smaller. Size here, however, is so variable that I do not lay much stress on this argument. In the third place, if the nuclei were dividing we should find dumb-bell shaped figures with the diameter of the nuclei drawn out at right angles to the plane of division. This is not the case, the minute nuclei remaining as spherical as though not in contact. In the fourth place we should expect to find connecting strands of chromatin substance between the recently divided karyosomes if it were a case of division, but no such connecting strands exist. In the fifth place we should expect to find the daughter karyosomes elongated in the axis at right angles to the plane of division if it were division. Such is not the case as inspection of the figures shows, while in many cases the two karyosomes are elongated in an opposite direction (Fig 2, *d*; Fig. 3, *c*). In the sixth place if it were division we should expect it to take place more rapidly than the figures indicate, for in fusion, the process in protozoa requires a longer time than does division and the large number of double forms of these secondary nuclei indicates that the process is a relatively slow one.

On the whole, therefore, I believe the evidence justifies no other conclusion than that this is a process of fusion and not of division and that we are dealing here with an actual conjugation of nuclei. The fusion takes place by preliminary union of the extreme peripheries, this is followed by union of the homogeneous portions, and finally by union of the karyosomes (Figs. 2, 3). In one or two cases I have seen some evidence that more than two nuclei may thus fuse. Fig. 2, *e*, for example presents such a case, the larger size, and the two karyosomes indicating that fusion of two nuclei has already taken place. It occurred to me that the minute nuclei, before fusing, might possibly divide in some form of maturation division, but I have been unable to confirm this supposition in the material at hand, and incline to the belief that fusion occurs at once, for the uniting nuclei are abundant in the immediate vicinity of disintegrating primary nuclei.

The result of this fusion of gametic nuclei is, in each case, a nucleus of somewhat larger size in which the central granule fragments into a cloud of extremely minute chromatin granules lying about a central space which is the beginning of the vacuole characteristic of the later phases in development of the spores (Figs. 9, 10, 14). These granules next collect in small aggregates which are arranged about the periphery of the vacuolated mass, from 70 to 100 of them, as nearly as I can estimate, being formed in each of the many centers which now correspond to those multiplication centers of sporozoa called sporoblasts by Schaudinn. In this period, the sporulating centers or sporoblasts, are carried about in the cytoplasmic flow and appear as small hollow nuclei which, in many cases, resemble the nuclei of some *Pelomyxa*-like forms. In the specimen from which Fig. 11 of my original paper was taken, there are more than 200 of these centers of multiplication, while in the encysted form shown in my original Fig. 12, a section of which is shown here in Figs. 13 and 14, there are about 250 of these sporoblasts.

The ultimate form assumed by the *Amœba* in the material which I possess, may be described as a collection of these sporoblasts, each one of which is a hollow sphere, the walls being studded with minute granular nuclei from 70 to 100 in number (Fig. 14). They lie about the one remaining primary nucleus which is shown in the section reproduced in Fig. 13.

A first examination of these nuclei in the sections gives the impression that the amœba body is well infested by parasites and this indeed, was my belief until a critical examination of the material in all stages. convinced me of my error. While under the belief that these nuclei were parasites I sought to interpret the several phases of the *Amœba* cycle which was described in 1904, as effects of such an infection. I concluded that minute parasites enter the body of *Amœba*, stimulate the nucleus to divide as does *Plasmodiophora brassicæ* the cell nuclei in the cabbage root, and then multiply by division in the interior of the endoplasm, the rapid multiplication filling the body with the minute granules which, earlier, I had interpreted as chromidium granules. My impression was strengthened by the observations of Schubotz[1] who interpreted the nuclei which I had described in *A. proteus*, as degenerating nuclei, an interpretation with which I quite agree, although not in the sense he meant. Prandtl[2] still more recently has published an interesting account of the development of young forms of *Allogromia* as parasites in the endoplasm of *Amœba proteus* and he also agrees with Schubotz that the nuclei described in my earlier paper are degenerating nuclei, and suggests that my "chromidium granules" may be young phases of an organism similar to the *Allogromia* which he describes.

While frankly admitting the possibility that these small nuclei may be parasites, a possibility which with fixed material and on morphological grounds alone, cannot be entirely refuted, I firmly believe that they are not parasites but developmental phases of *Amœba proteus*. My reasons for this belief may be briefly summarized as follows: First, the early stages of nuclear increase are present in specimens of *Amœba* in which there are none of the supposed parasites. Second, the supposed parasites must originate inside of the supposed degenerating nuclei, for, as Figs. 2, 5, 6, 7 and 8 clearly show, the structures under consideration first arise inside of the nuclei, and wander outside by dissolution of the nuclear membrane. Third, if they are parasites they must conjugate inside of the endoplasm of the *Amœba*, for, as we have seen above, there are no good grounds for interpreting these structures as dividing forms. Fourth if the structures in question are conjugating parasites the conjugation leads to further development within the protoplasm of the host cell without any protecting membranes against the resistance of the host cell. Fifth, if these things are parasites, then the secondary nuclei of *Arcella*, *Polystomella* and *Entamœba* must likewise be parasites, for the resemblance between the several cases is too strong to allow another interpretation. Finally, if they are parasites, they must wander into the nuclei of *Amœba proteus* in the form of germs too small to be recognized and grow there into larger nucleus-like bodies which emerge from the nucleus and conjugate, and all this without disturbing the physiological equilibrium of the *Amœba*, and without effecting any pathological change such as formation of vacuoles or spaces about themselves (cf. Figs. 2, 3, 4). The evidence, therefore, is altogether in favor of the nuclear character of these questionable structures, and their union, I believe, can be interpreted in no other way than as the fertilization of this universal rhizopod.

---

[1] "Beiträge zur Kenntnis der *Amœba blattæ* und *A. proteus*," *Arch. f. Prot.*, VI., 1905.

[2] "Der Entwicklungskreis von *Allogromia* sp.," *Arch. f. Prot.*, IX., 1907.

The fertilization of *Amœba proteus* has been sought for by biologists for decades. Many observations have been published on phenomena supposed to be conjugation processes, but these in the main, have turned out to be cases of plastogamy, common amongst the rhizopods, or cases of engulfing of one by another. If the fertilization were any ordinary process similar to what occurs in other allied forms, there seems little likelihood that it would have been overlooked for these many years. But occurring as I have now shown it to occur, in a manner quite unlike that of the majority of other rhizopods that we know, the reason for its being overlooked becomes apparent. Many have observed *Amœba proteus* in the multinucleate condition. Carter in 1863 observed as many as 70 nuclei in specimens of *Amœba princeps* which is usually regarded as the same as our *Amœba proteus*, and Wallich in the same year observed the liberation of many fine granular bodies by the rupture of the nuclear membrane, while Schaudinn[1] calls attention to the fact that he has observed the nuclear multiplication and suggests that it betokens a possible sexual phase.[2]

The process of fertilization, as I have described it here, comes under the head of the conjugation phenomena known as endogamy, or conjugation of nuclei within the original cell parent. It is not the only instance of such a phenomenon amongst the *Sarcodina*. Hertwig, in 1898, described self-fertilization in the case of *Actinosphærium*, while Schaudinn in 1903 (*loc. cit.*) described it in the case of *Entamœba coli*, the harmless commensal of the intestine. In both cases, however, the process is described as much more complicated than that which I have outlined here. In *Actinosphærium* the vegetative nuclei are reduced by fusion or by absorption to a relatively small number. The cell then divides into as many daughter cells as there are nuclei (five to ten); these daughter cells encyst within the parent cyst, the nucleus divides by mitosis and each of the cysts divides into two daughter cysts, each with one nucleus. The nucleus in each of these daughter cysts next divides twice, giving rise to two "polar body" equivalents. The cytoplasm and remaining nuclei of the two daughter cysts then fuse and the fertilization is completed by the reunion of the parts. In *Entamœba* the process is more like that of *Amœba* as described here. The cell throws out foreign matter and waste products of its own metabolism, and becomes smaller, more compact and more spherical. After secreting a gelatinous membrane in which the cell remains encysted, the cell nucleus divides into two nuclei, the division being followed by an incomplete division of the cell body. After this division, the daughter nuclei fragment, forming a mass of minute chromidial granules which are distributed throughout the cell. From the two masses of granules thus formed secondary nuclei arise by fusion as in the case of *Centropyxis*, *Arcella*, *Polystomella*, and rhizopods generally, and these two nuclei, after preliminary division giving rise to what Schaudinn interprets as "polar body" equivalents, divide for a third time, the products of this division fusing two by two. The partly separated protoplasmic body then reunites and the fertilization cell contains two fertilization nuclei. These two nuclei then divide twice forming eight nuclei altogether and these finally become the nuclei of eight amœboid spores.

---

[1] "Untersuchungen über die Fortpflanzung einiger Rhizopoden," *Arb. a. d. Kais. Gesundh.*, XIX., 1903.

[2] For further historical data see my original paper.

Except for the maturation divisions, which future study may reveal, the process of fertilization in *Amœba proteus* is thus strikingly similar to that of *Entamœba coli* the gametic nuclei arising by fragmentation instead of by division as in the latter case. The fertilization nucleus forms, not eight, but many daughter nuclei, and from analogy, I would expect these vacuolated centers of multiplication or sporoblasts in *Amœba proteus* to produce from 70 to 100 pseudopodiospores.

The formation of the secondary nuclei in *Amœba proteus* differs in some important respects from the process in other rhizopods. In *Arcella*, *Centropyxis* and *Polystomella* for example, it occurs in the chromatin substance that is either transfused through the membrane of the nucleus or formed by fragmentation of the nuclei. In all cases that have been worked out, however, the secondary nuclei are formed from the substance of this chromidium and we can thus trace their history back indirectly to the primary nuclei. In *Amœba proteus* on the other hand, the nuclei are not formed from diffused chromatin nor from the fragments of primary nuclei but they form directly within the primary nuclei and emerge from it as fully formed secondary nuclei. In this case, as in the other cases, the secondary nuclei are the gametic nuclei, only here their union is, so to speak, precocious and without the customary gamete formation, while the union and especially the further development *within the parent cell*, are unusual and unexpected discoveries.

Scheel takes up the life history of *Amœba proteus* from this stage on. In his paper on the Encystment of *Amœba proteus*[1] he describes compound cysts which I would interpret as a late stage (with protecting gelatinous membranes), of the stage shown in my Fig. 12 of the original paper (section shown in Figs. 13, 14 of present paper). Each one of his cysts I would interpret as one of the sporoblasts (Fig. 14), which, by independent growth, reaches the size he describes (about 80 microns). The peripheral granules (Fig. 14, *b*) of my sporoblasts become his nuclei of the cyst and later, the nuclei of the minute amœbæ with stellate pseudopodia (*Amœba radiosa*).

In conclusion I would substitute for the tentative life-cycle published three years ago, the following: The ordinary *Amœba proteus* reproduces asexually by division (seen in every laboratory); ultimately the asexual cycle is replaced by the sexual, the conditions of which, periods, etc., are entirely unknown; the sexual cycle is inaugurated by the multiplication through division of the nuclei until many "primary" nuclei are formed; these primary nuclei fragment directly into minute granular nuclei corresponding to the "secondary" nuclei of *Polystomella*, *Centropyxis*, etc. In *Amœba* the secondary nuclei may be called the "gametic" nuclei; the gametic nuclei fuse to form fertilization nuclei; in these the fused karyosomes fragment to form finely divided chromatin (it is, strictly speaking, not a chromidium for it is entirely intra-nuclear), while a vacuole forms in the interior; this vacuolated fertilization nucleus becomes a center of multiplication (equivalent in every way to a sporozoön sporoblast); by accumulation of these fine chromatin granules the peripheral or "tertiary" nuclei are formed; the tertiary nuclei, surrounded by a minute bit of plasm, grow into the pseudopodiospores observed by Scheel (hypothetical); these young pseudopodiospores break away from the parent cyst and develop into young amœbæ formerly known as *Amœba radiosa*, and these, in turn, develop into the ordinary *Amœba proteus* of pond and laboratory.

Columbia University,
New York City, August 10, 1907.

---

[1] Festschrift für Carl von Kupffer, Jena, 1899.

EXPLANATION OF PLATE XI.

The photographs are from sections, stained with iron-hæmatoxylin, of the same specimens of *Amœba proteus* that were pictured in my previous paper on "Evidences of a Sexual-cycle in the Life-history of *Amœba proteus*. The magnifications vary from 550 diameters (Fig. 13) to 2,000 diameters (Figs. 9 and 10).[1]

Fig. 1. Part of section of *Amœba* with about 30 primary nuclei some of which have begun to fragment. The chromatin is massed in a characteristic manner about the periphery while the small points in the center are the karyosomes of the future secondary nuclei. At this stage there are few secondary nuclei in the cytoplasm. × 1,000.

Fig. 2. Section from the amœba represented in Fig. 7 of my previous paper. The majority of the primary nuclei have fragmented and the cell body is spotted with the secondary nuclei. These may be seen *forming* in the large primary nucleus (*f*). The photograph also shows many stages in the fusion of the secondary nuclei. At (*a*) two are seen with the peripheries in contact; at (*b*) the bodies are beginning to fuse; at (*c*) and (*d*) fusion of bodies is completed, while the karyosomes have not yet fused but lie facing one another (if this were division the karyosomes would be elongated in the direction at right angles to this); at (*e*) there is an apparent double fertilization; at (*g*) the karyosome from the upper nucleus has migrated into the lower nucleus before fusion of the nuclei is complete. × 1,500.

FIG. 3. Primary nucleus with brood of young secondary nuclei within it and several secondary nuclei in various stages of fusion (*a*), (*b*) and (*c*) representing different stages in the process. × 1,350.

FIG. 4. Primary nucleus with most of the secondary nuclei gone. Some secondary nuclei below in different stages of early fusion, while above are two later stages of union. × 1,500.

FIG. 5. Primary nucleus in the process of liberating secondary nuclei. Two fully formed secondary nuclei are passing into the cytoplasm at (*a*), while others not yet formed remain in the nucleus. Fig. 7 is a deeper section of the same primary nucleus showing the fully formed secondary nuclei within it. × 1,500.

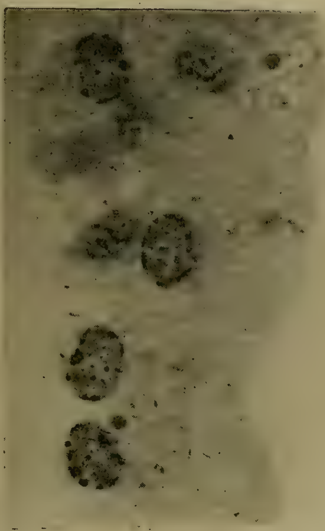
FIG. 6. Section of an amœba in which all the primary nuclei (*a*), (*b*), (*c*) are fragmenting and disintegrating while the secondary nuclei are in various stages of fusion. The section was injured by the objective so that some of the pairs of secondary nuclei are spread. × 1,500.

FIG. 7. Section of same nucleus as that shown in Fig. 5, showing brood (*a*) of secondary nuclei not yet liberated. × 1,500.

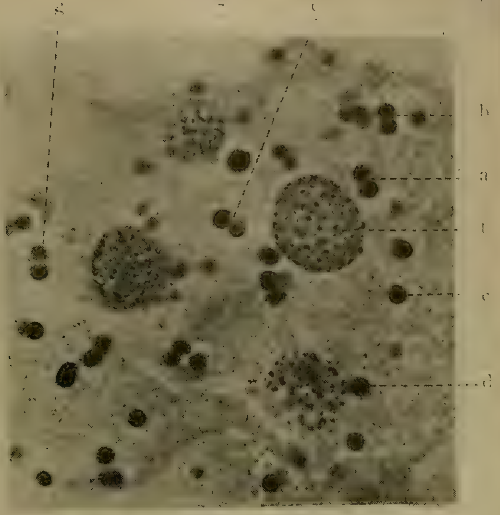
---

[1] The actual dimensions in the photographs must be increased by one fifth to give the magnifications stated.

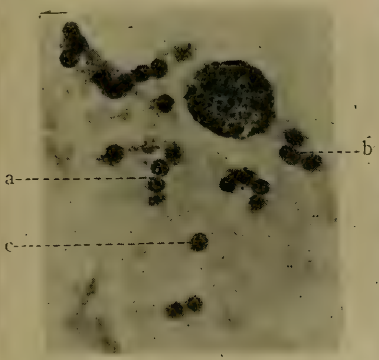
1



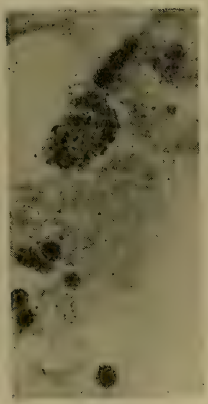
2



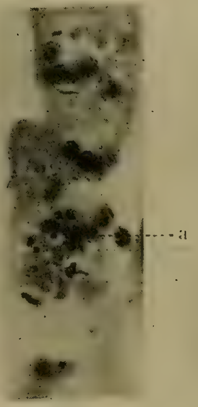
3



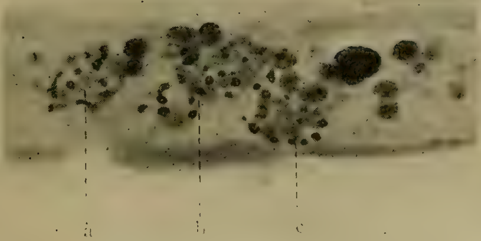
4



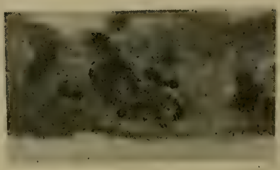
5



6



7



Calkins

EXPLANATION OF PLATE XII.

FIG. 8. Two primary nuclei showing broods of secondary nuclei (*a*).

FIGS. 9 and 10. Development of the fertilized nuclei, two photographs of the same section at slightly different foci. The upper nucleus at (*a*) shows the characteristic vacuole which becomes the vacuole of the later stages (cf. Figs. 13 and 14). The karyosomes fragment into minute chromatin granules which can be seen in Fig. 9. × 2,000.

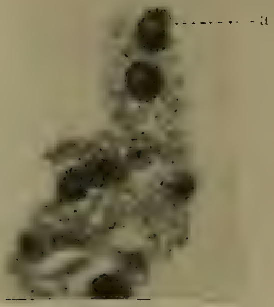
FIG. 11. Later stages in development of the fertilized nuclei; the chromatin granules from the disintegrated karyosome now form accumulations about the periphery, these, later, form the nuclei of the spores. × 1,400.

FIG. 12. Intermediate stages in development between that shown in Fig. 9 and that of Fig. 11.

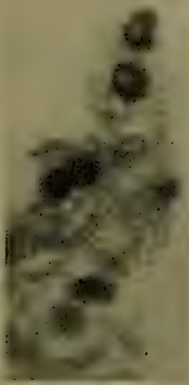
FIGS. 13 and 14. Sections of the amœba pictured in Fig. 12 of my earlier paper in the stage of encystment. In Fig. 13 (× 550) the primary nucleus shown is the residual nucleus comparable to the primary residual nucleus in the case of *Polystomella*. Here it is surrounded by many sporoblasts, which, in Fig. 14, are shown more highly magnified (× 1,500). At (*a*) and (*b*) the small but perfect peripheral nuclei may be clearly seen.

8

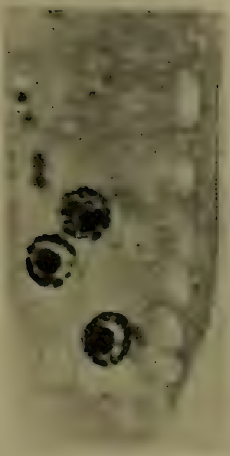
9



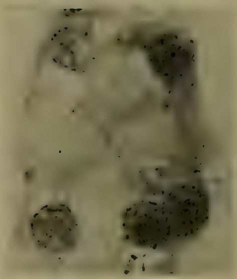
10



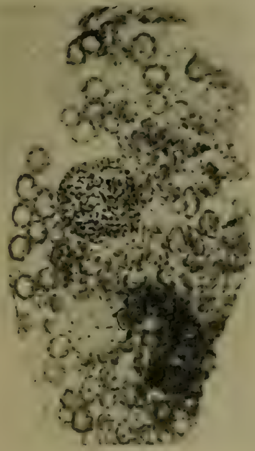
11



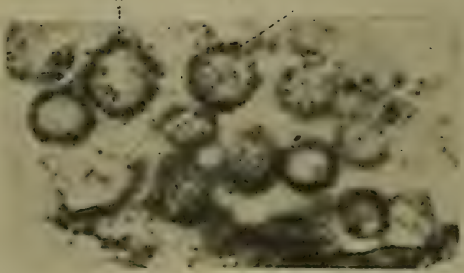
12



13



14



Calkins

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

VOL. XIII OCTOBER, 1907 No. 5

CONTENTS

		PAGE
BLOUNT, MARY.	*The Early Development of the Pigeon's Egg, with Especial Reference to the Supernumerary Sperm Nuclei, the Periblast and the Germ Wall*	231
PATTERSON, J. THOS.	*On Gastrulation and the Origin of the Primitive Streak in the Pigeon's Egg.—Preliminary Notice*	251
MORGAN, T. H., AND STOCKARD, C. R.	*The Effects of Salts and Sugar Solutions on the Development of the Frog's Egg*	272
GREGORY, L. H.	*The Segmental Organ of Podarke obscura*	280
STOCKARD, C. R.	*A Peculiar Legless Sheep*	288

Smithsonian Institution
National Museum

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY
PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN	AGENT FOR GERMANY	AGENT FOR FRANCE
WILLIAM WESLEY & SON	R. FRIEDLÄNDER & SOHN	LIBRAIRIE ALBERT SCHULZ
28 Essex Street, Strand *London, W. C.*	*Berlin, N. W.* *Carlstrasse, 11*	*3 Place de la Sorbonne* *Paris, France*

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter,
under Act of Congress of July 16, 1894.

Editorial Staff.

E. G. Conklin—*The University of Pennsylvania.*

Jacques Loeb—*The University of California.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*American Museum of Natural History, New York.*

C. O. Whitman—*The University of Chicago.*

E. B. Wilson—*Columbia University.*

Managing Editor.

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Holl, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa.

Vol. XIII. *October, 1907.* *No. 5.*

BIOLOGICAL BULLETIN

THE EARLY DEVELOPMENT OF THE PIGEON'S EGG, WITH ESPECIAL REFERENCE TO THE SUPERNUMERARY SPERM NUCLEI, THE PERIBLAST AND THE GERM WALL.

MARY BLOUNT.

A PRELIMINARY PAPER.

In the *American Journal of Anatomy*, September, 1904, there appeared a paper by Dr. E. H. Harper on "The Fertilization and Early Development of the Pigeon's Egg." Dr. Harper found that the egg is polyspermic; that one sperm nucleus unites with the egg nucleus; and that the supernumerary sperm nuclei migrate to the periphery of the germinal area and there set up an accessory cleavage. He followed through the development to the sixteen-cell stage, or about eight hours after fertilization, and although he gives two figures of sections of an egg fifteen hours after fertilization, the intervening stages were not filled in.

At the zoölogical laboratory of the University of Chicago, in January, 1905, I took up the study of the pigeon's egg, hoping to continue from the sixteen-cell stage. But in order to appreciate the material, it was necessary to go back into earlier stages. I have obtained an egg for every hour of development from the formation of polar bodies to the time of laying — a period of about forty-one hours. For some of the more critical stages before laying I have more abundant material, and also have a good many laid eggs.

The purpose of this preliminary paper is to announce some of the more important steps in the early development, but without presenting the abundant proof which the material affords for my conclusions.

Through the kindness of Prof. C. O. Whitman and other members of the Department of Zoölogy, I have been the recipient of a university fellowship which has enabled me to pursue this study. Dr. F. R. Lillie, at whose suggestion I undertook the research, has followed the work carefully, and I thank him for his interest and kindness. I am also indebted to Mr. W. L. Tower for his help in photography. The living egg is a difficult subject, and it was only after a great many efforts that I secured any photographs. Twelve cleavage stages have been photographed, although only three are presented in this paper.

METHODS.

Following the method of workers who have preceded me, the blastoderm has been killed and hardened on the yolk, and the orientation marked with a bristle: Immediately after a window has been made through the shell, a bristle is inserted in the side of the yolk toward the blunt pole of the shell. Later (usually when the egg is in 70 per cent. alcohol) a five-sided piece, including the blastoderm, is cut out from the yolk. One side of the five-sided area is perpendicular to the chalazal axis, and is toward the large pole of the egg. Two sides are parallel to each other and to the chalazal axis, and the last two sides meet in a sharp angle pointed toward the small pole of the egg. Fig. 1 makes this orientation clear, the anterior side of the blastoderm being toward the point of the arrow. This five-sided block is

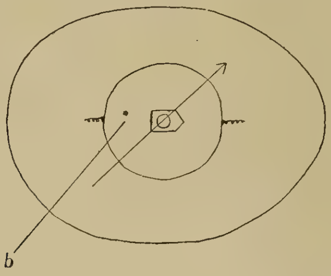


FIG. 1. Diagram to show the method of marking the orientation. The arrow indicates the direction of the axis of the future embryo. *b*, bristle.

easily seen in the paraffin cake for orientation in cutting. Kleinenberg's picro-sulphuric acid (strong solution) plus 10 per cent. acetic has been the most successful for killing and fixing, although other solutions have been used.

FERTILIZATION.

Dr. Harper (3) found that the egg is fertilized in the evening, at the time it leaves the ovarian capsule and enters the oviduct and he makes this statement, "In all cases observed, this has taken place between seven and nine o'clock." I shall, therefore, refer to eight o'clock in the evening as the hour of fertilization, although the exact time for any particular oviducal egg is not known.

To present the history of the early development, I shall describe several critical stages as follows:

1. Position of the supernumerary sperms at the close of maturation.
2. The 8-cell stage.
3. The 16-cell stage.
4. The last stage of the multiplication of the sperm nuclei.
5. The disappearance of the sperm nuclei.
6. The periblast.
7. The growth of the blastodisc at the expense of the periblast.
8. The germ wall.

1. *Position of the Supernumerary Sperm Nuclei at the Close of Maturation.*— In an egg taken from the oviduct at 11:30 P. M., or about three and one half hours after fertilization, the first cleavage plane had not formed. In this egg, the supernumerary nuclei had migrated into the periblast at the periphery of the germinal area, and they occupied a circle which in later stages is indicated superficially by accessory cleavage. Some of these nuclei were in mitotic division.

2. *The 8-cell Stage.* — Abundant material has been obtained in stages of two and four cells, but for brevity, a description of those stages is omitted from this paper.

At 4:45 A. M., eight and three fourths hours after fertilization, an egg of eight (or perhaps nine) cells was taken from the shell gland. Its surface view is shown in Fig. 2, and a transverse section in Fig. 3. The dotted circle, Fig. 2, represents the distance to which the sperm nuclei may migrate peripherally (it is also the peripheral limit of the periblast nuclei, to be explained later), but the accessory cleavage, with a few exceptions, is confined to the zone just outside the blastomeres of the primary area. A migrating sperm nucleus is shown at the extreme right of Fig. 3. Another sperm-nucleus has migrated under the large blastomere *a*. It is in the central periblast, which will be explained later. A study of the whole series of sections showed a number of nuclei in this position, forming a submarginal circle. They migrate later as far centrally as the margins of the nucleus of Pander, but were never found under the very center of the blastoderm.

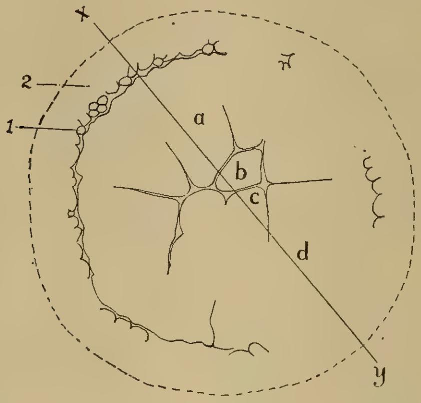


FIG. 2. Sketch of surface view of a pigeon egg about eight and three fourths hours after fertilization, 4:45 A. M. *a*, *b*, *c*, *d*, cells of primary cleavage which are shown in section in Fig. 3. *xy*, the plane of the section in Fig. 3. 1. Accessory cleavage. 2. Periblast.

Fig. 3 is of a section taken through the plane *xy* of Fig. 2.

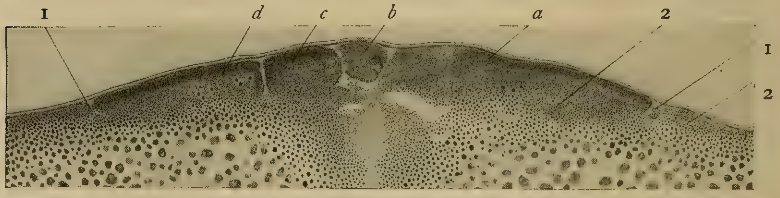


FIG. 3. Transverse section of the pigeon egg whose surface view is shown in Fig. 2. *a*, *b*, *c*, *d*, cells of primary cleavage. 1. Accessory cleavage. 2. Migrating sperm nuclei.

The cells of this section will be recognized as the cells with the corresponding letters in Fig. 2. The small cell *b* is cut off from the underlying yolk in this section, but it is so only at its central end. In sections anterior to this, the cell *b* is continuous with the yolk. The split made by this horizontal cleavage marks the position of the *future segmentation cavity.* This horizontal plane may not be permanently established at this stage. It seems to come and go during the next few hours of development. But *its position indicates the depth of the center of the blastoderm in cleavage stages.*

A comparison of the several stages here represented (Figs. 3, 5, 6 and 9) by actual measurement of the drawings will show that there is but the slightest variation in the depth of the germinal disc at the center.

In contrast with this, is the account of the hen's egg by Kölliker (6). He describes the blastodisc as increasing in depth as cleavage progresses. In his Fig. 19 (which represents a vertical section through a hen's egg of about twenty cells) two central "*Furchungskugeln*" and two marginal "*Segmenten*" are shown; *i. e.*, there are four cells in the section forming a single layer. Between this layer of cells and the white yolk is the unsegmented "*Bildungsdotter.*" None of these products of cleavage is completely cut off from the "*Bildungsdotter.*" They form a layer .14 mm. in depth in the center. A section through a later stage in the development of the hen's egg is shown in Kölliker's Fig. 22 where, "*die Dicke der durchfurchten Stelle in der Mitte des Keimes gerade noch einmal so dick war, als in dem früher beschreibenen Fälle (Fig. 19) nämlich 0.28–0.30 mm.*" . . . "Somit greift die Durchfurchung, indem sie weiterschreitet, in der Mitte der Keimschicht immer mehr in die Tiefe, wie schon Oellacher dies vermuthet hat, und erreicht am Ende nahezu die Grenze der Lage die in der Fig. 19 mit *bd* als ungefurchten Bildungsdotter bezeichnet ist."

Kölliker suggests that the adding of cells from below may be by a process similar to the adding of cells to the central part from the marginal segments, — *i. e.*, the nucleus of a marginal segment divides and the central end of the segment containing one of the daughter nuclei is cut off and becomes a "Furchungskugel." The other daughter nucleus passes into the marginal segment, and so on until finally the part of the marginal segment left over, changes over into a "Furchungskugel." And so, according to Kölliker, the first appearing *Furchungskugeln* are never completely cut off from the unsegmented *Bildungsdotter* below, but nuclei, sisters to those in the first layer of cells, pass down into the *Bildungsdotter*. Here nuclear division takes place, and cells are organized around the upper daughter nuclei, thus forming the second layer of cells in the center of the blastodisc, while the lower daughter nuclei are left deeper in the "Bildungsdotter." And thus cleavage proceeds downward until finally the last remaining nucleated portions of the "Bildungsdotter" change over into "Furchungskugeln."

In the pigeon's egg, on the contrary, I do not find any such deepening of the center of the blastodisc. The change from one layer to several layers of cells is by a process *exactly like that of the teleost egg*. See Agassiz and Whitman (1) Fig. 2, and Wilson (8) Figs. 16, 17, 18 and 19. *The blastodisc of the pigeon's egg becomes stratified by horizontal cleavage planes arising above the first horizontal cleavage; i. e., above the level of the plane which limits the cell b below* (Fig. 3). *Nuclei are never found in the central part below the level of the horizontal cleavage under the cell b.*

Extending deep into the white yolk is a cone of slightly granular protoplasm. It varies in extent in different stages as will be seen by comparing Figs. 3, 5, 6 and 9. A more central section than Fig. 5 shows this cone extending deeper. In some stages it is better described as being funnel-shaped, with the slender tube of the funnel going deep into the yolk, and the mouth opening on the the lower side of the blastodisc. In some of the sections of the egg represented in Fig. 9 it is found at twice the depth of the figure. If the sections were cut exactly perpendicular to the surface, the funnel would appear continuous from its broad end at the blastodisc to the deepest limit included in the section. A similar structure has been figured by Eycleshymer (2) for the egg of *Lepidosteus osseus*, Figs. 32, 34 and others. He calls it, "the peculiar conical prolongation of the periblast."

3. *The 16-cell Stage.*—Fig. 4 is a photograph of a pigeon egg of sixteen cells. Although of later development, it was obtained an hour earlier than the egg shown in Fig. 2,—*i. e.*, it was taken from the oviduct at 3:45 A. M., seven hours and forty-five minutes after the time from which fertilization is reckoned. The arrow indicates the direction of the axis of the embryo, and the anterior side of the blastoderm is in the direction of the point of the arrow.

Three principal regions in the blastoderm of the bird's egg are to be recognized in surface view at this stage: (1) the *central area*, (2) the *marginal cells*, (3) the *periblast*. In this egg (Fig. 4), the central area is occupied by six cells [the Furchungskugeln of Kölliker (6)] and there are ten marginal cells (Kölliker's Segmenten). The periblast is the zone outside the marginal cells. At the inner margin of this zone is the accessory cleavage caused by the supernumerary sperm nuclei. This is on all sides, but at a few places where there are no sperm nuclei, the large marginal cells are open peripherally.

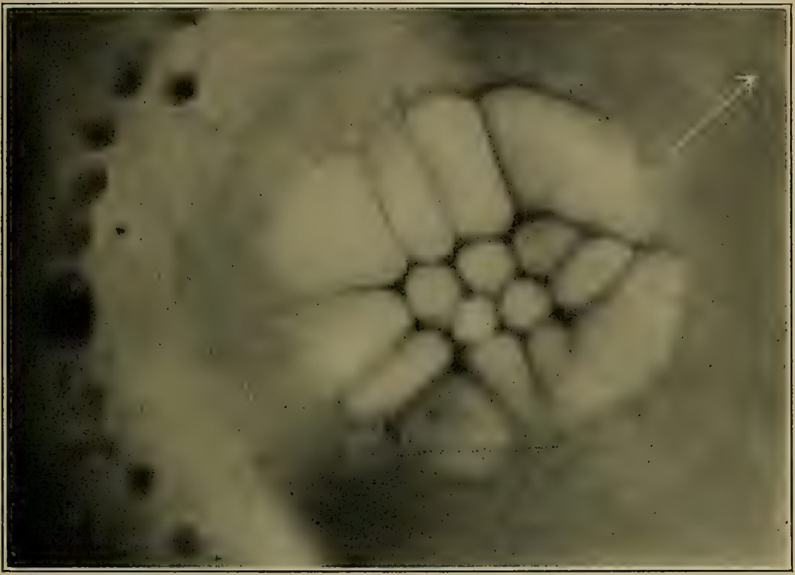


FIG. 4. Photograph of a pigeon's egg 7¾ hours after fertilization. 3.45 A. M. The anterior side of the blastoderm is toward the point of the arrow.

As cleavage proceeds cells are cut off centrally from the marginal cells, and added to the central area, and thus the latter grows at the expense of the former (compare Fig. 7). Radial cleavage planes divide the marginal cells and increase their number, while the central cells are constantly becoming smaller by division. Finally, the marginal cells are all used up, and we recognize only two regions in the blastoderm, (1) the central area, and (2) the periblast. In early stages, all of the cells are continuous with the yolk, but as development proceeds, the central cells become complete below and separate from the yolk, and only the marginal cells are open below. Thus the marginal cells constitute a "zone of junction" (see Agassiz and Whitman (1), Figs. 2, 3, 4 and 5) between the segmented and unsegmented parts of the egg. All of the photographs presented in this paper show a very symmetrical form of cleavage, and while I have found a good many instances of asymmetrical cleavage, I cannot agree with Kölliker (6) that "Die Furchung geht immer asymmetrisch vor sich, so dass ohne Ausnahme die eine Hälfte der Keimscheibe in der Zerklüftung der anderen voran ist."

4. *The Last Stage of the Multiplication of the Sperm Nuclei.* — In an egg obtained at 6:30 A. M., ten and a half hours after fertilization, the sperm nuclei were very numerous. There is no record of the exact number of cells of primary cleavage showing on the surface of this egg, but there were a few more than thirty-two. The accessory cleavage was very abundant and more than one cell in depth. A transverse section through about the cen-

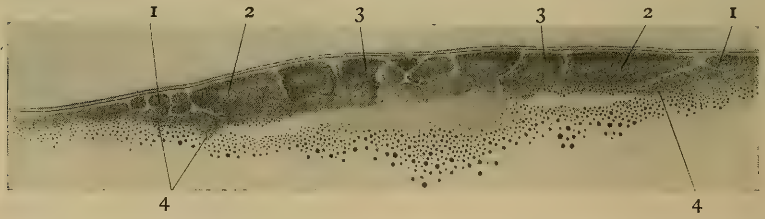


FIG. 5. Transverse section of a pigeon's egg at the end of the period of multiplication of the sperm nuclei. Egg taken 6.30 A. M., about 10 hours after fertilization and 31 hours before laying. Note that all cells are still continuous with the yolk. 1. Accessory cleavage around the sperm nuclei. 2. Marginal cells sharply separated from the sperm nuclei. 3. Central cells. 4. Sperm nuclei.

ter of this blastoderm is shown in Fig. 5. The accessory cleavage is confined to the region immediately outside of the large marginal cells of the blastoderm, but the sperm nuclei have migrated peripherally into the unsegmented part. These nuclei were more abundant than this drawing suggests; for on the right hand side of the section there were four more nuclei in superficial positions in the unsegmented part beyond the limits of the figure. The sperm nuclei were just as abundant in every other section of this egg. But these nuclei have migrated not only peripherally; they are also under the large marginal blastomeres. The latter, however, are definitely separated from the sperm nuclei by cleavage planes whose significance will be better appreciated in contrast with a stage after the disappearance of the sperm nuclei as shown in Figs. 6 and 9.

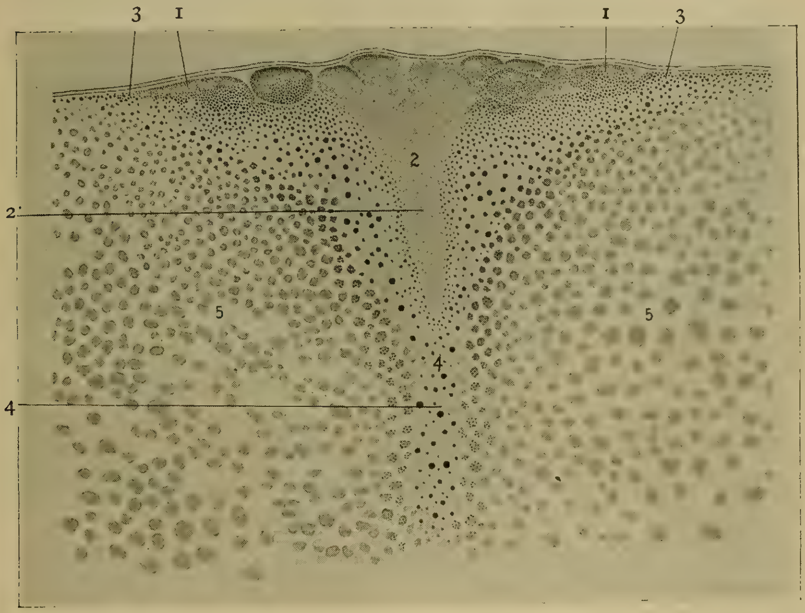


FIG. 6. Longitudinal section of pigeon's egg at the time of disappearance of the sperm nuclei; on the left (anterior), the marginal cell has become open, *i. e.*, continuous with the marginal periblast. On the right the marginal cell is still slightly separated from the periblast at the surface. Surface view of the egg showed traces of accessory cleavage; note continuity of the central cells with central periblast. 1. Marginal cells. 2. Cone of protoplasm. 3. Marginal periblast. 4. Neck of latebra (white yolk). 5. Yellow yolk. Egg taken 7 A. M., about eleven hours from fertilization (estimated).

5. *The Disappearance of the Sperm Nuclei.*— Fig. 6 represents a longitudinal section through about the center of the blastoderm of an egg taken from the oviduct at 7:00 A. M., or eleven hours after the approximate time of fertilization. There were in this egg a few remaining sperm nuclei, and where they occurred, they were separated from the marginal cells by cleavage planes similar to those on the right of Fig. 5. In sections where the sperm nuclei did not appear, the marginal cells were open to the *periblast,* as on the left of Fig. 6. On the right of this figure (which is the posterior side of the blastoderm) the marginal cell is partly closed in, and in a few sections beyond this, it was entirely closed, being separated from a cell of accessory cleavage. In surface view, also, the marginal cells were open peripherally except where the accessory cleavage occurred.

In another egg taken from the bird at 7:00 A. M. (eleven hours from fertilization) *every marginal cell as seen in surface view was open peripherally, and in sections, the margin was like that at the left of Fig. 6. Not one nucleus was found outside the cells of primary cleavage.*

Other eggs of about this period show accessory cleavage on the wane and conditions in sections like those in Fig. 6. In the egg represented in Fig. 5, the sperm nuclei were fragmenting. They disappear between ten and twelve hours after fertilization.

Fig. 7 is a photograph of an egg eleven hours from fertilization (7:10 A. M.). Here, the central, marginal and periblastic regions are clearly expressed. This is probably a stage after the disappearance of the sperm nuclei, and nearly all of the marginal cells are open peripherally. At the posterior side there are suggestions of accessory cleavage. These small cells are probably mere bud-like projections from the periblast, and not due to the presence of sperm nuclei. It is impossible to decide this point from surface view, but sections would show the relations between the marginal cells and the periblast, and therefore demonstrate whether the nuclei of these small cells were derived from supernumerary sperms or from the cleavage nucleus.

6. *The Periblast.*— Previous paragraphs have anticipated the discussion in this. Any mention of the periblast refers the student of vertebrate embryology to the work of Agassiz and Whitman

(1) on *Ctenolabrus* where the origin of the periblast was first accurately described. Only the twelve marginal cells of the sixteen-cell stage of the teleost egg rest upon the yolk. The contact with the yolk is at the inferior outer angle of the cells, and this region "may be designated as the zone of junction" (Agassiz and Whitman) between the blastodisc and the periblast. The marginal cells of the teleost are open peripherally. Now, there is to be recognized in the bird's egg a periblast exactly comparable at this stage (eleven or twelve hours after fertilization) with the periblast of the fish egg. We may think of a *potential periblast* in the *unsegmented pigeon's* egg. Into this the sperm nuclei migrate.

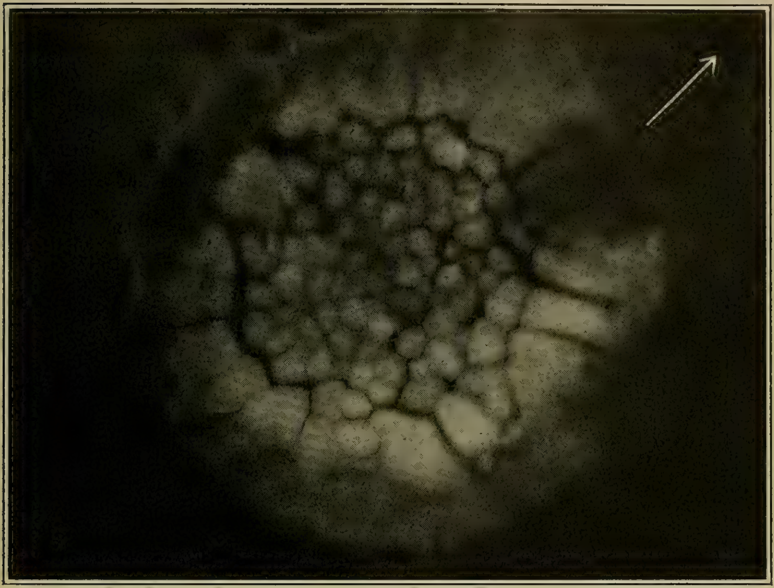


FIG. 7. Photograph of pigeon's egg 11 hours after fertilization, 7.10 A. M. The point of the arrow indicates the anterior side.

After these nuclei disappear the marginal cells of the blastodisc open peripherally to the periblast and are directly continuous beneath with the yolk. The nuclei of the marginal cells divide, and some of the daughter nuclei migrate into the unsegmented region, and thus the periblast "becomes cellular," to use the expression of Agassiz and Whitman (1). The periblast nuclei migrate peripherally and also into subgerminal positions, and thus we may speak of a *marginal* and *central* periblast. But the nuclei of the central periblast have not been found in the nucleus of Pander.

In *Lepidosteus osseus*, Eycleshymer (2) found the nuclei most numerous at the center, "undergoing rapid division and contributing one derivative to the cell cap.

Fig. 8 is a photograph of an egg obtained at 9:30 A. M., or thirteen and a half hours after fertilization. The marginal cells are now limited peripherally, but are open below as is suggested in Fig. 9, a transverse section through another egg of about the same age. The periblast in such an egg as Fig. 8 is demonstrated only in sections. It does not appear in surface view.

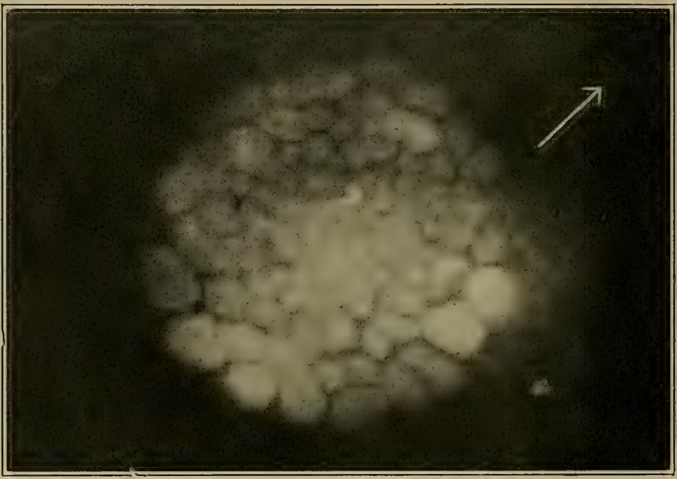


FIG. 8. Photograph of pigeon's egg 13½ hours after fertilization, 9.30 A. M. Anterior side of blastoderm toward point of arrow.

7. *The Growth of the Blastodisc at the Expense of the Periblast.* — In the teleost egg, after the conclusion of cleavage, the periblast remains distinct from the blastodisc, but in the pigeon's egg, the periblast continues to add cells to the segmented region. I have studied this point carefully up to several hours after laying, but have not completed my study on later stages of incubation. To illustrate this, there is a series of drawings, Fig. 9 to Fig. 15.

The transverse section represented in Fig. 9 is through about the center of the blastoderm of an egg taken from the bird at 10:30 A. M., or about fourteen and a half hours after fertilization. The center of the blastodisc has become three cells deep, and is separated from the yolk by a sharp line bounding the latter. But the marginal cells are continuous with the yolk, and protrusions from the central periblast extend into the segmentation cavity. Nuclei are often found in these protrusions, which suggest that cells are being added to the segmented part. This egg is still in the cleavage stage, being twenty to twenty-two hours before gastrulation [considering the time of gastrulation five to seven hours before laying as determined by Mr. Patterson (7)], and may therefore be considered not unlike the teleost egg. But in following through the successive later stages, similar relations are found between periblast and blastodisc and there is no time when they are distinct.

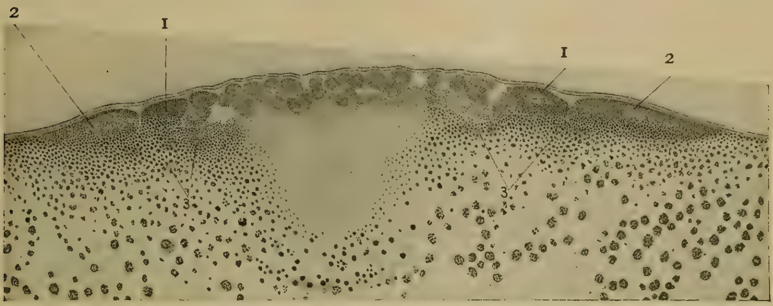


FIG. 9. Transverse section through the center of the blastoderm of a pigeon's egg taken at 10:30 A. M., 14½ hours after fertilization. 1. Marginal cell. 2. Marginal periblast. 3. Nuclei in the central periblast, derived from the nucleus of the marginal cell.

Fig. 10 shows in mere outline the conditions at the margin of

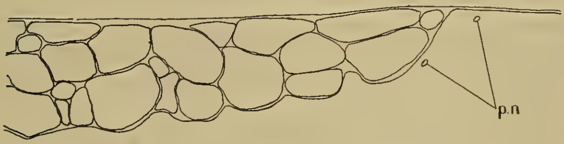


FIG. 10. Margin of a transverse section of a pigeon's egg about twenty and a half hours after fertilization, 4:25 P. M. *p.n*, periblast nuclei.

the blastoderm at 4:25 P. M., or twenty and a half hours after fertilization. Some of these cells at least have been derived by division of such a marginal blastomere as shown in Fig. 9. Others may have been derived from the periblast, with nuclei sisters to those yet remaining in the unsegmented part.

Fig. 11 is the posterior end of a longitudinal section through an egg perhaps twenty-five hours after fertilization (8:50 P. M.) Four nuclear nests and two single nuclei are found in the periblast. Beyond the limits of the drawing, are four other nuclei two of them are in line with the most extreme nucleus to the left and two are a little deeper. Large masses, as shown at 3, Fig. 11, are organized out of the periblast and subsequently they divide into smaller cells. Indentations just to the left of the segmented part here suggest future cleavage which would add superficial cells. (Compare Fig. 14.) This figure (Fig. 11) resembles Harper's (3) Fig. 36 which is a section of an egg fifteen hours after fertilization. Harper considers that the "free nuclei" are sperm nuclei but there was a gap in his material just at the period when the sperm nuclei disappear and the periblast is organized. The nuclei of his Fig. 36 are doubtless periblast nuclei.

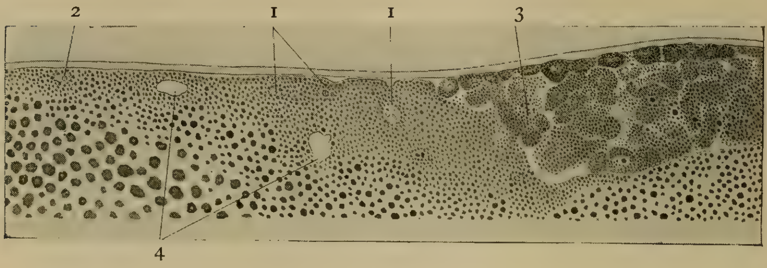


FIG. 11. Posterior side of a longitudinal section of a pigeon's egg about twenty-five hours after fertilization, 8:50 P. M. 1. Nests of periblast nuclei. 2. Periblast nucleus. 3. Syncytial mass derived from the periblast, organizing into cells which will be added to the blastodisc. 4. Vacuoles.

Fig. 12 shows a marginal part of a horizontal section through an egg of the same age as Fig. 11 (twenty-five hours after fertilization, 8:50 P. M.). Here are "free nuclei" or periblast nuclei and such relations between the segmented and unsegmented part as to suggest contribution of cells from the periblast. Of course, such segregation of cytoplasm and granules around nuclei as is indicated at 2 and 3 may not be permanent. The nucleus for the cell at 3 is in the next section. Amitotic division is suggested by some of the nuclei. Compare the position of nuclei in a syncytial zone outside the segmented blastodisc with the condition shown in Wilson's "Embryology of the Sea Bass" (8), Figs. 23 and 24.

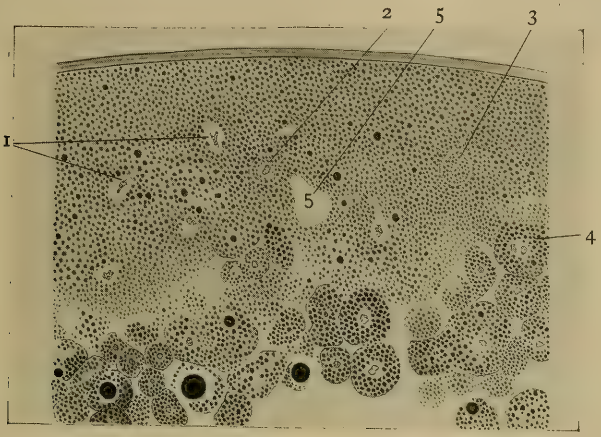


FIG. 12. Marginal part of a horizontal section through a pigeon's egg about twenty-five hours after fertilization, 8:50 P. M. 1. Periblast nuclei. 2 and 3. Cells being organized out of the syncytium. The nucleus for 3 is in the next section. 4. A cell contributed from the periblast. 5. Vacuole.

The following outline drawings, Figs. 13, 14, and 15, need little explanation. They are of transverse sections of eggs about 26, 28 and 32 hours respectively after fertilization. They sug-

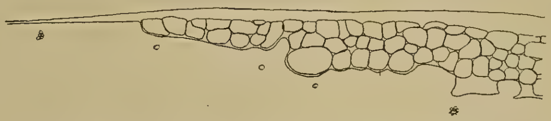


FIG. 13. Margin of a transverse section of a pigeon's egg, about 26 hours after fertilization. Notice cells being added to the segmented part from the periblast. The periblast nuclei were not all in this section, but were found in four successive sections. Two nuclear nests are shown.

gest the spreading of the blastoderm over the unsegmented part by cells organized around the superficial periblast nuclei. The blastoderm instead of having an almost perpendicular margin as in Fig. 10, comes to lie over the periblast. These figures show other additions of cells besides those at the extreme margin.

8. *The Germ Wall.* — The term *Keimwall* was first used by His in 1866. In his description of the germ wall of the hen's egg, His (5) says that in the first hours of incubation the white yolk on which the border of the germ area rests is grown through with cells of the germ, and it forms a peculiar structure with protoplasmic frame work enclosing white yolk spheres. To this structure, His gave the name "*Keimwallgewebe*" or *organisirten Keimwall.*

His (5) also says that he was able to follow "wie tiefliegende Zellen des Keimes vermöge ihre sehr ausgesprochenen amöboiden Beweglichkeit die ihnen benachbarten Dotterkörner und Dotterkugeln in sich aufnehmen."

In another paper His (4) says, "Während der ersten Zeit der Bebrütung entsendet die untere Schicht jenes Randtheils Fortsätze zwischen die Elemente des Keimwalles, so dass diese grossentheiles in ein Gerüst archiblastischen Protoplasmas eingeschlossen werden."

This conception of His is based upon a study of hen's eggs during the first few hours of incubation. He makes no reference to the germ wall in the unlaid egg. A study of the pigeon's egg in close stages of development before laying gives quite a different conception of the germwall, — particularly as to the origin of the nuclei. They are *periblast nuclei,* and are not derived from the "tiefliegende Zellen des Keimes." Such nuclei are shown in Fig. 16 which represents the margin of the blastoderm in transverse section of a pigeon egg six hours before laying. It is, of course, a younger stage of the germ wall than is

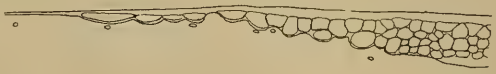


FIG. 14. Margin of transverse section of a pigeon's egg about 28 hours after fertilization. The periblast nuclei, except the most peripheral one, were all in this section.

represented in any of the figures by His (5). Moreover, none of his figures through the germ wall of the hen's egg show the extreme margin of the blastoderm. The nuclei in the unsegmented part in Fig. 16 are periblast nuclei, and their history can be traced back through each preceding stage of development to a period about eleven or twelve hours after fertilization when nuclei from the marginal cells pass into the periblast. Indeed, such a history of the nuclei may be retraced through the figures of this paper — Figs. 16, 15, 14, 13, 11, 10, 9, 6.

As development proceeds from such a stage as is represented in Fig. 9, the zone of junction established by the marginal cells between the blastodisc and the periblast travels outward and the blastodisc increases in diameter as cells are added to its margin from the periblast. Cells are organized around the superficial periblast nuclei and sisters to these nuclei are left deeper in the unsegmented periblast. Thus the extreme margin of the blastodisc is thin, Figs. 13, 14 and 15. But later, the deeper sister-nuclei are enclosed in cells and so the blastodisc thickens up under that part which had been only one layer of cells in depth. But, meantime, the *thin margin* has advanced over the yolk, by addition of cells from the periblast. However, there comes a time a few hours before laying (Fig. 16) when the *margin* thickens up. This, I think, is the condition described by His (5), "Am umbebruteten Keim sind die Zellen der unteren Keimschicht von denen der oberen nicht allzusehr verschieden. In dem Randtheil eines unbebrüteten Huhnerkeimes gehen obere und untere Keimschicht in einander über und sie sind nahezu gleich dick. Die untere, lockerer gefügt als die obere, ist eher etwas schwächer. . . . Dotterkörner finden sich auch in Zellen der obern Schicht, obwohl nicht sehr reichlich."

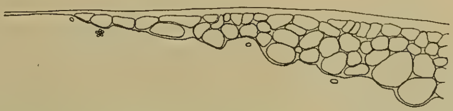


FIG. 15. Margin of a transverse section of pigeon's egg about 32 hours after fertilization. The periblast nuclei were all in this section. One nuclear nest is shown. Notice additions from the periblast to the segmented part.

In other literature it is said that the *lower germ layer* forms a *compact mass* with the *germ wall,* which, like a thickened border, rests upon the yolk. This thickened border also receives the name *Randwulst* and *bourrelet blastodermique.*

I would describe the margin of the blastoderm (Fig. 16) not as a region where the *upper and under germ layers go over into each other, but as a syncytial region out of which two layers of cells differentiate centrally.*

Through each period of development leading up to this stage (as is shown by the series of figures in this paper), the periblast nuclei keep ahead of the advancing margin of the blastodisc. They multiply and a part of them are used up in the cells that are continually being added to the margin. Such a nucleus in *advance* of the margin of the blastodisc is shown at the left of Fig. 16. It is in the periblast. It is not enclosed in a cell — *i. e.,* it is not separated by a cleavage plane from the periblast which extends further peripherally — other periblast nuclei are *below* the margin. The thickened-up character of the margin is due to the *upward differentiation* of cells from the periblast. It is not a region where the blastodisc is deepened by the opening of the lower layer of cells to send protoplasmic processes into the white yolk. The cells of this region, which are open below, are so *because they have not yet, in the process of their differentiation out of the periblast, become closed.* Large nucleated masses differentiate *upward* from the periblast. These masses become multinucleate, and finally divide up into several cells. As this region becomes older, that is, as it is left behind while the margin of the

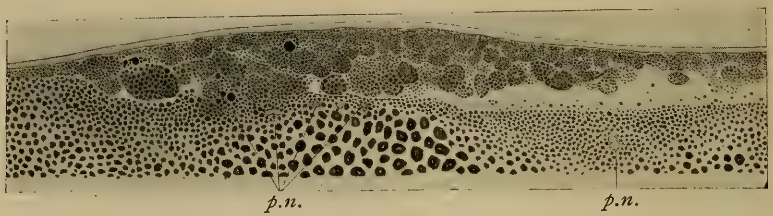


FIG. 16. The germ wall in transverse section through the center of the blastoderm of a pigeon's egg, 8:10 A. M., 36 hours after fertilization and 6 hours before laying. *p.n,* periblast nuclei. They were not all found in this section, but were reconstructed from five successive sections. There are six other periblast nuclei in this half of the section, but in positions central to the limits of the figure. The right hand side of the figure is toward the center of the blastoderm.

blastoderm advances, the cells individualize and separate from each other. The whole thickened margin is a *syncytium.* It is an *embryonic region* whose depth is measured from the *vitelline membrane* to the lowest limit of the periblast. There is not an ectoderm extending over this region to the extreme margin of the blastoderm. The cells next to the vitelline membrane, are, of course, flattened against it, but their lower border does not give the character of an epithelial layer. Only on the side of this syncytium toward the center of the blastoderm do the cells next the vitelline membrane form a true epithelium. From this thick, syncytial border region cells *individualize centrally*, and form two layers — (1) an upper layer, the ectoderm, and (2) a lower layer of loosely arranged cells.

It is not difficult to explain the presence of yolk-granules in the cells of the upper layer because these cells, like all others in this region are derived from the periblast in which the yolk-granules are abundant.

The section whose margin is shown in Fig. 16 presents two layers of cells in the central part, which roof over a large cavity filled with fluid. The marginal part of the cavity is shown in the figure. Periblast nuclei are everywhere under the blastodisc except in the nucleus of Pander. From them are derived the nuclei which are, in later stages of incubation, in the germ wall of the area opaca (His) and in the Randwulst. I shall not at present attempt to discuss these later stages, but in a more complete paper, I shall present further evidence in support of this conception of the germ wall and shall describe it in other than transverse sections, and shall also describe the formation of the vascular layer.

SUMMARY.

1. The supernumerary sperm nuclei migrate into the potential periblast and disappear between ten and twelve hours after fertilization.

2. The position of the cleavage cavity is indicated by the first horizontal cleavage.

3. After the disappearance of the sperm nuclei, the marginal cells open peripherally and the periblast becomes organized with nuclei derived from the cleavage nucleus.

4. Cells are added to the blastodisc from the marginal and central periblast.

5. The "free nuclei" under the blastodisc are periblast nuclei. They are the nuclei of the germ wall, including the *Randwulst.*

LITERATURE.

1. **Agassiz and Whitman, C. O.**
'84 On the Development of some Pelagic Fish Eggs. Preliminary Notice. Proc. Amer. Acad. Arts and Sciences, XX., 1884.

2. **Eycleshymer, A. C.**
'03 The Early Development of Lepidosteus Osseus. The Decennial Publications, Univ. of Chicago, X., 1903.

3. **Harper, E. H.**
'04 The Fertilization and Early Development of the Pigeon's Egg. Am. Jour. Anat., Vol. III., No. 4, pp. 349–386, Sept., 1904.

4. **His, Wilhelm.**
'75 Der Keimwall des Hühnereies und die Entstehung der parablastischen Zellen 1875. Zeitschrift für An. u. Entwgesch., Bd. I., S. 274 ff.

5. **His, Wilhelm.**
Lecithoblast und Angioblast der Wirbelthiere. Abhandlungen der Königl. Sächs. Gesellschaft der Wissenschaften. Math.-Phys., XXVI.

6. **Kölliker, Albert von.**
'79 Entwicklungsgeschichte des Menschen und der Höheren Thiere. Leipzig, 1879.

7. **Patterson, J. Thos.**
'07 On Gastrulation and the Origin of the Primitive Streak in the Pigeon's Egg. — Preliminary Notice. Biol. Bull., Oct., 1907.

8. **Wilson, H. V.**
'91 The Embryology of the Sea Bass (Serranus Atrarius). Bull. U. S. F. C., Vol. 9, pp. 209–277 (1891).

ON GASTRULATION AND THE ORIGIN OF THE PRIMITIVE STREAK IN THE PIGEON'S EGG. — PRELIMINARY NOTICE.

J. THOS. PATTERSON.

The results of the experimental studies of Assheton ('96), Miss Peebles ('98), and Kopsch ('02) on the primitive streak of the chick demonstrate beyond any reasonable doubt that the material of that structure enters into the formation of the embryo — a view long held by many embryologists. In the light of these experiments the opposite view of Balfour and his followers is no longer tenable. The results obtained by these three workers have, in the main, solved the problem of the fate of the primitive streak, but they have not answered the question of its origin. The present work was undertaken with the hope of throwing light upon the latter question. It was soon found that its solution depended upon a morphological and experimental study of stages occurring before the time of laying. The morphological results mainly will be considered in this paper.

Material and Methods.

It is doubtful if a more desirable material could be found for the purposes of this investigation than that furnished by the pigeon's egg. The regularity of the laying habits of the common pigeon makes it possible to secure eggs at approximately any stage of development. Breeders have long known that this bird ordinarily lays two eggs at a sitting, the first usually between four and six P. M., and the second between one and two P. M., on the second day following. According to Harper ('04) this latter egg is fertilized at about eight P. M., just before it enters the oviduct, and hence it is forty-one hours in traveling down this passage. Thus, it will be seen that the investigator can secure this second egg at approximately any stage of its early development, for he needs but kill the bird at the proper hour and remove the egg from the oviduct in order to obtain a desired stage. Eggs removed in this manner, even as early as twenty hours before laying, can be used for experimentation; for by this time the shell is firm enough to permit handling without injury to the blastoderm.

In dealing with this material it was necessary to use special technique both for fixing and orientation. The picro-sulphuric-acetic mixtures have been found to be vastly superior to all other reagents. Of these mixtures the most successful is 92 parts of Kleinenberg's strong picro-sulphuric plus 8 parts of glacial acetic. The whole yolk is immersed in this fluid for one hour and is then treated with 70 per cent. alcohol for several hours, after which it is placed in 80 per cent. At this point it is found advisable to cut out a properly oriented wedge-shaped block of yolk containing the blastoderm, with vitelline membrane still attached. After completely washing out the picric acid, this block is carried through the higher alcohols, cleared in cedar oil, and embedded and sectioned in the usual way.

For stages prior to the appearance of the primitive streak, such a treatment necessitates a careful orientation of the blastoderm before using the fixing fluid. Already a method for orienting the chick blastoderm has been worked out. Thus a number of investigators have shown that if a hen's egg be held in front of the observer so that the blunt end is to his left and the pointed end to his right; the posterior margin of the blastoderm will be towards and the anterior away from him, and hence, when the embryo appears, its head will be directed away from the observer, with its long axis meeting the chalazal axis at right angles. If a pigeon's egg be held in a similar position a different condition is found. The posterior margin of the blastoderm, instead of being directly in front of the observer, is forty-five degrees to his left, and when the embryo arises, its long axis meets the short axis of the egg at an angle of forty-five degrees (see Fig. 1).

For some purposes iron hæmatoxylin has been of great value as a stain, but for general use a modification of Delafield's hæmatoxylin is unsurpassed, especially for demonstrating the presence of cell walls.

GASTRULATION.

I stated above that it was necessary to investigate the period of development that occurs before laying. A study of these early stages naturally involves the question of the origin of the two primary germ layers. Concerning the manner in which these two layers arise there has been a wide difference of opinion among embryologists, although a great deal of attention has been paid to this question. The unsatisfactory solution of this problem is due to the fact that most of the conclusions are based on incomplete evidence. So far as I am aware, not a single observer has had a complete series of normal stages of any one type from which to draw his conclusions. The divergent views as to the origin of the entoderm, however, can be grouped into three classes. (1) A number of the older workers have maintained that it arises by a process of delamination, that is, the upper cells of the segmented disc arrange themselves into a continuous layer, constituting the primary ectoderm, while the deeper cells of the disc form the primary entoderm. This view is not in accord with what is known to occur in many other forms. (2) Others have maintained that the entoderm arises by an ingrowth of cells into the segmentation cavity from a part or all of the inner edge of the germ-wall. The most recent contribution supporting this view is by Nowack ('02) who states that the bulk of the entoderm is formed out of a mass of cells, which grows forward from the posterior part of the germ-wall. In speaking of this forward growth he says: "Es gehen nämlich von der Gegend des hinteren Keimwalles, als unmittelbare Fortsetzung desselben, kurze Zellstränge aus, die mitten durch die Keimhöhle nach vorn ziehen, miteinander in Verbindung treten und eine dünne Platte von verschiedener Dicke und vielen grösseren und kleineren Löchern bilden. Diese Platte endet vorn und an den Seiten mit freiem,

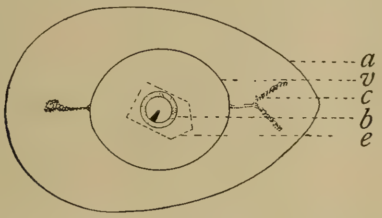


FIG. 1. Scheme for orienting the blastoderm of the pigeon's egg in cutting sections. *a*, shell; *b*, blastoderm at the first appearance of the primitive streak; *c*, chalaza; *v*, vitelline membrane; *e*, wedge-shaped block of yolk containing the blastoderm which is cut out and embedded for sections.

wenn auch unregelmässigem Rande, zeigt also ein zungenförmiges Aussehen. Man wird wohl nicht fehlgehen, dieses Gebilde als den Anfang der unteren Keimschicht, d. h. des Entoderms zu bezeichnen. Es ist dies allerdings nicht das einzige zellige Material, was innerhalb der Keimhöhle zu finden ist, aber doch die bei weitem grösste Menge."[1] (3) The third class includes those who believe that the entoderm arises by a process of gastrulation, that is, the upper layer turns under to give rise to the lower layer. This view has been supported by Haeckel, Goette, Rauber, and others. The work of Duval ('84) also has been quoted in support of gastrulation. This author describes the blastoderm at the end of segmentation as a biconvex lens (lentille biconvexe), in which two layers can be recognized ; an upper epithelium-like layer separated by a narrow fissure from a thick lower layer. The deepest cells of the latter are open below to the white yolk of the Nucleus of Pander. In a later stage a thickening occurs on the margin where the upper layer is united with the lower. Duvals calls this thickened rim the *bourrelet blastodermique.* It corresponds to the *Randwulst* of the German authors. At the posterior margin where the rim is thickest, a crescent-shaped groove appears, which passes forward beneath the blastoderm as a fissure separating the lower cells of the blastoderm from the underlying yolk. Duval now regards the blastoderm as in the gastrula stage and hence the fissure between the yolk and the thick lower layer is the archenteron. It is clear that, in the main, Duval's theory is one of delamination. So far as the pigeon's egg is concerned the segmentation cavity is *not found* just below the superficial layer of cells at the end of segmentation, but is situated beneath the central portion of the blastoderm — between the deepest cells and the yolk.

In order to work out the history of a continuous developmental process, such as gastrulation, it is necessary to have a complete series of normal stages taken from one type. Such a series is easily obtainable from the pigeon, and the following account of gastrulation is based upon a study of several series of this birds's egg.

In seeking for a stage at which to begin the account of gastru-

---

[1] *Loc. cit.*, p. 27.

lation, I found the close of segmentation to be the most advantageous time, for it is shortly after this period that the first direct steps leading up to invagination occur. At the close of segmentation the disc is three or four cells deep, except at the extreme margin where it gradually diminishes to a thickness of one or two cells. Beneath and external to the marginal cells of the disc, yolk or "periblastic" nuclei are present. According to Miss Blount ('07) these nuclei segregate about themselves the neighboring protoplasm, and later, cell walls appearing are added to the disc, thus contributing to its extension. Waldeyer ('69), Hertwig ('99), and others have advanced similar views for the chick and selachian, designating it supplementary cleavage. Where these cells are being added to the disc a more or less syncytial condition exists around the entire margin. This region constitutes the germ-wall.

Shortly after the period described above there occurs the first direct step in the process of gastrulation. This is in the nature of a thinning of the posterior part of the segmented disc. This process begins, not at the extreme margin, but usually slightly posterior to the center, and then spreads in all directions, but with more rapidity towards the posterior margin.[1] The first stage of this process is shown in Fig. 2. Slightly posterior to the center and almost directly above the segmentation cavity, the disc is but two cells deep, while in the region of the germ-wall it is four deep. The characteristic features given above for the germ-wall and the extension of the disc can also be made out from this figure. At this time the segmentation cavity is still very shallow, but upon further progress of the thinning out it becomes much more extensive, and may then be called the subgerminal cavity.

As the thinning out progresses the germ-wall becomes interrupted in the posterior region, as is shown in Fig. 4. The blastoderm from which this drawing was made is much more advanced than that in Fig. 2, being about eleven hours older. The changes occurring between these two stages, however, are gradual and may be followed with comparative ease. In the first place there is a very rapid division of cells, as is evidenced by

---

[1] *In Torpedo ocellata* Zeigler ('02) describes the thinning-out of the blastoderm as beginning at the posterior and progressing anteriorly.

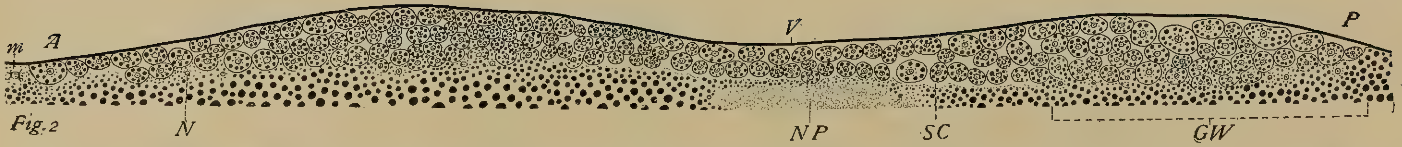


FIG. 2. A median longitudinal section of a blastoderm taken twenty-one hours after fertilization, or twenty hours before laying. *m*, a periblastic nucleus in the process of division. × 77.3.

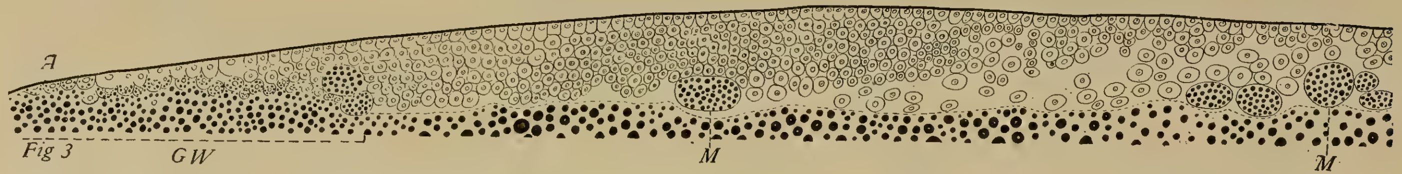


FIG. 3. Anterior half of a median longitudinal section of a blastoderm taken thirty-one hours after fertilization, or ten hours before laying. × 129.5.

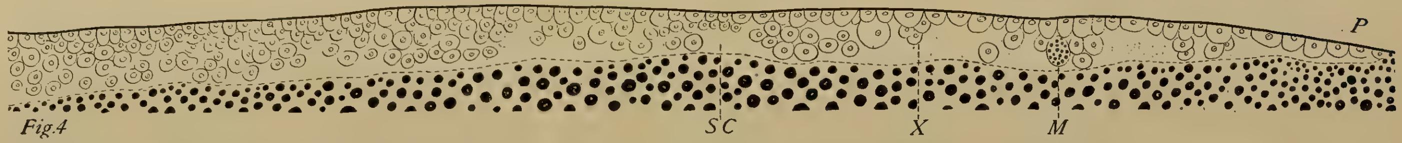


FIG. 4. Posterior half of the same section as represented in FIG. 3. This figure shows the condition of the posterior part of the blastoderm just before invagination begins. *X*, cells crowding into the ectoderm. × 129.5.

their number and size in the anterior half of the blastoderm (Fig. 3), where they are about seven layers deep. However, as one passes from the anterior to the posterior margin there is a gradual change in depth from seven cells to one. There are also found in the anterior region large yolk masses (Fig. 3, *M*), which arise from the floor of the segmentation cavity. At this stage they are not limited to this region, but occasionally are found in the posterior half (Fig. 4, *M*), where the disappearance of the germ-wall is one of the most characteristic features. This interruption of the germ-wall goes hand and hand with the thinning out, which is rapidly establishing a one-layered condition of the blastoderm. In other words the phenomenon of thinning-out is nothing more nor less than the crowding of the cells of the segmented disc into a single layer. It is evident that this must result in a rapid centrifugal expansion of the blastoderm. That this is actually the case is shown by measurements. Thus at twenty hours after fertilization the average diameter of the blastoderm is 1.915 mm., while at thirty hours it is 2.573 mm. In fact there is no other period in the early history of the blastoderm in which there is such a rapid increase in the surface area, as occurs during the time when the thinning out is at its maximum. One would not be justified, however, in saying that this entire expansion is brought about by the thinning out, for according to Miss Blount's interpretation the germ-wall is also contributing materially to this increase.

In the posterior third of this blastoderm the single layer is almost complete; still, at places, some of the few cells yet remaining in the segmentation cavity can be seen apparently in the act of crowding up into the single layer (Fig. 4, *X*). Whether or not, in all cases these remaining cells eventually succeed in getting into the upper layer, approximately above where they are situated is not clear. They do in the majority of blastoderms, but I have some few series in which they seem to migrate anteriorly and to the sides, where the last stages of thinning out occur. In either case they take no part in the formation of the gut-entoderm.

In Fig. 5 is shown a reconstruction from sections of the blastoderm represented in Figs. 3 and 4. The germ-wall does not completely encircle the rim of the blastoderm, but it is interrupted for a distance of about 70 degrees at the posterior margin. The form of the germ-wall is that of a crescent, with its horns stopping slightly short of the area over which a single layer has been developed.

During the two or three hours immediately following the stage just described, the thinning out continues to extend anteriorly over the central portion of the blastoderm, and at the same time spreads laterally. By the time the posterior third of the blastoderm is thinned out to approximately one layer there occurs the initial step in gastrulation. Owing to the individual variation in the development of eggs, some difficulty has been experienced in securing a complete series through this brief period, so that at present I am not in a position to state positively in what this initiatory step consists. The evidence, however, inclines me to believe that it is brought about by a rolling under of the thin

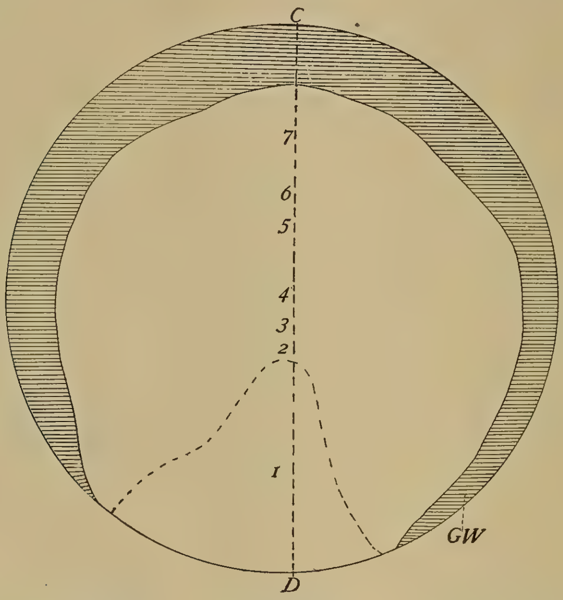


FIG. 5. A diagrammatic reconstruction from sections of the blastoderm from which Figs. 3 and 4 were drawn. *GW*, germ-wall. Numbers 1, 2, 3, etc., represent the regions of the blastoderm which are one, two, three, etc., cells deep, respectively. The broken line around 1 indicates the region where the blastoderm is approximately one cell deep. × 27.2

free edge of the posterior margin of the blastoderm. This interpretation is in harmony with what is known to occur in the gastrulation of other forms, especially the fish. Thus Agassiz and Whitman ('84) state that in *Ctenolabrus* "there is a plain rolling under, or involution, as an initiatory step in the formation of the ring," but they believe that it is more correct to describe the process "as an ingrowth, due both to a rapid multiplication of the cells, and also to the centrifugal expansion of the ectoderm." There are certain differences between the teleost and pigeon blastoderms which, in this connection, must not be overlooked. Thus at the time of invagination the teleost blastoderm is three or four cells thick, and the epidermal layer of the ectoderm takes no part in the involution. On the other hand, the pigeon blastoderm is approximately but one cell thick at the posterior margin where invagination occurs, and hence all the cells of this margin participate in the involution. The interpretation of sections certainly supports this view, but the appearance of sections is often misleading. Two other sources of evidence are much more convincing. In the first place, careful measurements show that previous to and following gastrulation the blastoderm is nearly circular, but during the period of invagination the antero-posterior diameter is *always shorter* than the transverse diameter. This is exactly what one would expect if the posterior margin turns under instead of growing out over the yolk. In the second place an injury made on the posterior margin of the blastoderm during the early stages of invagination is found, upon further incubation, in the gut-entoderm, that is, it has been carried down under the blastoderm.

There occurs simultaneously with the turning under of the free edge a rapid thickening in the region of invagination, that is, on the posterior border where the upper layer turns under to become continuous with the invaginated portion. This thickening is not to be accounted for merely by a multiplication of cells *in situ*, but is largely brought about by a movement of material to the median axis from the lateral portions of the posterior margin of the blastoderm. This shifting of material necessarily brings about the approximation of the horns of the germ-wall, and in thus approaching each other they finally meet, and thus close the blastopore. All this will become clear upon examining a blastoderm during the period in which gastrulation is at its height, and comparing it with a stage such as is shown in Fig. 5.

Fig. 6 is a reconstruction of a blastoderm at the height of gastrulation, which was taken five hours before laying, or thirty-six hours after fertilization. It represents a dorsal view as though the ectoderm were transparent. On the anterior and lateral margins is a clear crescent-shaped area (*O*), the region of over-growth. Between this area and the subgerminal cavity (*PA*) is the germ-wall, in which two distinct zones can be recognized. The outer of these (*Z*) may be designated as the *zone of junction*,[1] and is

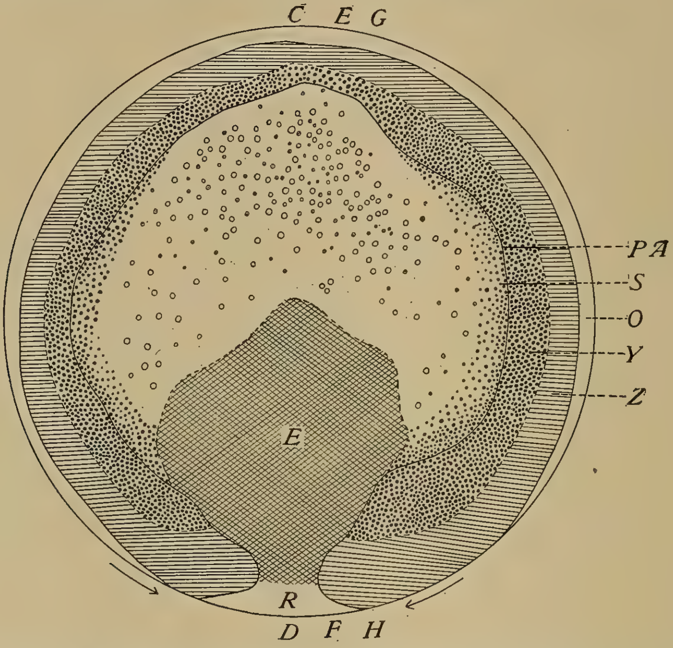


FIG. 6. A diagrammatic reconstruction of a blastoderm taken thirty-six hours after fertilization, or five hours before laying. It represents the ectoderm as transparent. *O*, region of overgrowth; *Z*, zone of junction; *Y*, yolk zone; *PA*, outer boundary of the area pellucida; *S*, beginning of yolk-sac entoderm; *E*, region covered by invaginated or gut-entoderm; *R*, dorsal lip of the blastopore. Lines drawn through *CD*, *EF*, and *GH* represent the planes of the sections illustrated in Figs. 8, 9 and 10, respectively. The arrows at posterior margin indicate the direction of movement of the halves of the margin. × 27.2.

---

[1] This term was first used by Agassiz and Whitman, '84.

characterized by a fusion of layers, in which there may be a more or less syncytial condition. Above the region of the inner or *yolk zone* a distinct ectoderm is present, below which are found many large cells heavily laden with yolk. These cells, which may be more or less surrounded by yolk, in which numerous "periblastic" nuclei are present, are destined to become the yolk-sac entoderm. This zone is the region formerly occupied by the zone of junction.

Within the subgerminal cavity are important structures. Scattered over the greater part of its area, but more numerous in the anterior region, are many large yolk masses, among which are also a few of the remaining segmentation cells that have not yet succeeded in getting into the ectoderm. At the extreme sides of the cavity are a great number of cells (*S*), which in the main are the same as the latter. However, it is possible that some of these may have been given off from the inner edge of the germ-wall. *E*, a tongue-like process, is the region over which the invaginated entoderm extends. It reaches from near the posterior margin to slightly beyond the center of the blastoderm. Its anterior and antero-lateral margins end freely, but its postero-lateral margins are bounded by the horns of the germ-wall. At the extreme posterior the entoderm is in connection with the thickened rim, a region of indifferent structure. Where the entoderm arises from the rim it is necessarily thick but it gradually thins out anteriorly. Beneath this rim (*R*) is a passage, the blastopore, which is widest at the margin, gradually narrowing as it passes toward the center. This passage becomes continuous with the cavity under the entoderm — the archenteric cavity.

Sections of this blastoderm are very instructive. A portion of the anterior half of a section, four sections to the left of the median line, is shown in Fig. 7. At the extreme anterior margin is the region of overgrowth (*O*), where the blastoderm first spreads over the yolk. The width of the overgrowth never exceeds that shown in this series, for as fast as it extends out over the yolk the germ-wall keeps pace with it. In the more peripheral portion of the germ-wall separate layers cannot be distinguished. This is the zone of junction, which seldom has a greater width than is shown in this figure. But next to the subgerminal cavity a distinct ectoderm can be recognized, and just beneath this ectoderm are cells mingled with yolk granules in which periblastic nuclei are present. As the overgrowth proceeds over the yolk, followed by the zone of junction, it is evident that that portion of the germ-wall between the zone of junction and the subgerminal cavity (*SG*), that is, the yolk zone (*Y*), will continue to increase in width. But the cavity, due to the liquefaction of yolk, is also increasing in width, but at a slower rate. In this widening of the cavity there are left around its margin cells which were previously embedded in the yolk. These cells form the beginning of the yolk-sac entoderm, and when the invaginated entoderm has spread over the subgerminal cavity its free margin becomes continuous with that of the yolk-sac entoderm. The last place for this union to occur is in the anterior region of the cavity.

Within this region of the cavity are found many large yolk masses (*M*), in some cases so numerous as to cause an elevation of the ectoderm, especially in later stages. Against the under surface of this layer are crowded a few remaining segmentation cells (*X*). These are the cells which Gasser ('82) has mistaken for wandering entoderm cells and Nowack ('02) for wandering ectoderm cells. At the extreme right of this figure the free end of the invaginated entoderm can be seen (Fig. 7, *L*).

Fig. 8 is a portion of the posterior half of a longitudinal median section (see Fig. 6). In the posterior region is the thickened rim, or dorsal lip of the blastopore (*R*).[1] From its rounded appearance one might infer that the posterior margin is still rolling under to form the entoderm, but at this stage it is more correct to regard the entoderm as arising as a forward growth from the inner edge of the thickened rim. At the place of origin (*U*) the entoderm is very thick, but gradually thins out anteriorly. The blastopore (*B*) is a shallow passage extending inward from the exterior to become continuous with the archenteric cavity (*AC*), which is that portion of the subgerminal cavity covered by the entoderm. Within the archenteric cavity are two large yolk masses (*M*) which have just risen out of the yolk. Below the

---

[1] The ventral lip is represented by the yolk lying immediately beneath the blastopore.

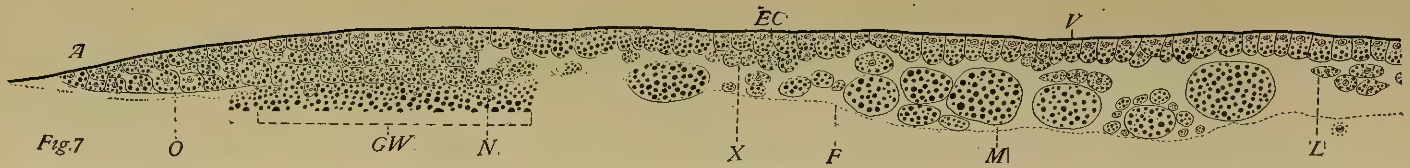

A
EC
V
Fig.7
O
GW
N.
X
F
M
L

FIG. 7. Anterior portion of a section taken slightly (4 sections) to the left of the plane passing through *CD* of Fig. 6. *X*, segmentation cells crowding into the ectoderm. × 169.

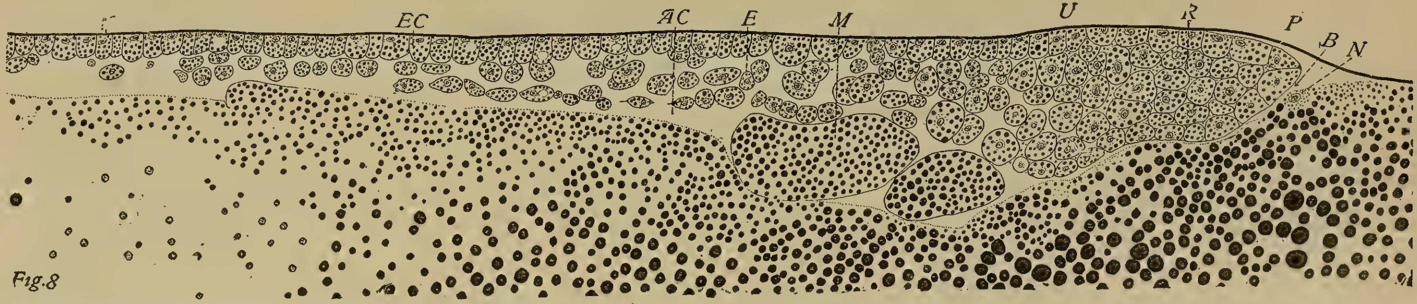

EC
AC
E
M
U
R
P
B
N
Fig.8

FIG. 8. Posterior portion of a median section taken through *CD* of Fig. 6. *B*, blastopore; *R*, dorsal lip of the blastopore; *U*, union between the deeper cells of the dorsal lip and the entoderm. × 169.

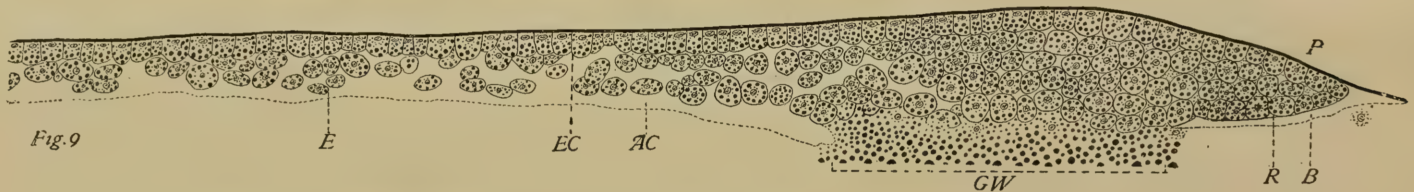

P
Fig.9
E
EC
AC
GW
R
B

FIG. 9. Posterior portion of a section taken through *EF* of Fig. 6. It shows the lateral portion of the lip (*R*) and of the blastopore (*B*). × 169.

blastopore no periblastic nuclei are present, except a few at the extreme margin of the blastoderm (*N*).

Fig. 9 represents the posterior portion of a section taken through *EF* of Fig. 6. At the extreme right is the dorsal lip of the blastopore (*R*). This lateral part of the lip is not so thick as in the median section (Fig. 8, *R*). Below the lip is the lateral portion of the blastopore. The section also passes through the end of the right horn of the germ-wall (*GW*). The remaining structures are very similar to the corresponding parts of Fig. 8 and need no further description.

From Fig. 6 it will be seen that a section taken through *GH* would no longer contain any portion of the blastopore, since in this region the outer edge of the germ-wall reaches to the margin of the blastoderm. This section is represented in Fig. 10, and its most important part, consisting of a mass of cells from which the entoderm arises anteriorly, is shown at *D*. Between this mass and the inner edge of the germ-wall there is a space in which only a few cells are present. In some sections, however, no such space exists, but the mass of cells is directly continuous with the germ-wall. In such cases it is easy to gain the impression that the mass is a part of the germ-wall, and thus to be led astray into concluding that the entoderm arises directly from the germ-wall. A close study, however, shows that the character of this mass, even in cases of its most intimate union with the germ-wall, is such as to make it easy to distinguish the one from the other. As previously stated, many of the cells in the region of the germ-wall are directly open to the underlying yolk, in which periblastic nuclei are present, but the cells of the mass are entirely separated from the yolk and are completely delimited by cell-walls. The significance of this mass will be considered in connection with Fig. 13.

Fig. 11 is a median longitudinal section of a blastoderm taken thirty-eight hours after fertilization. It shows the condition of the blastoderm shortly after the closing of the blastopore, and clearly represents the character of the entoderm during the few hours immediately following this event. It will be seen that the entoderm is not a continuous layer, especially in the anterior region, where the cells are more or less in groups. Later, how-

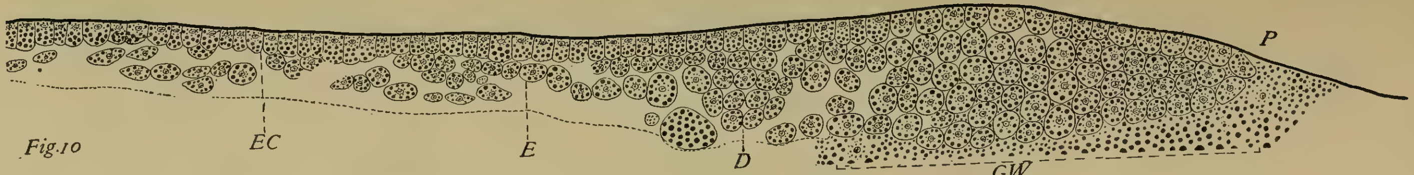
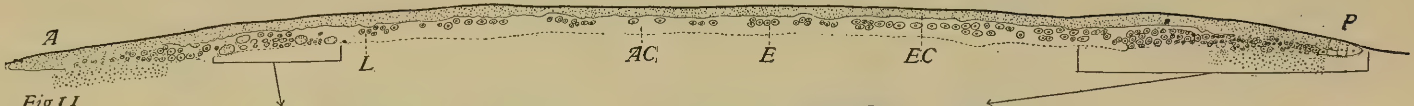
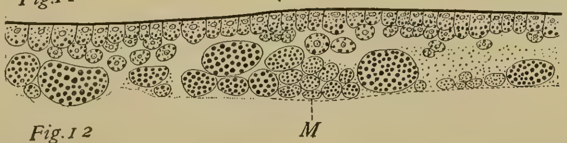


Fig.10
EC
E
D
GW
P

FIG. 10. Posterior portion of a section taken through *GH* of Fig. 6. *D*, mass of cells. × 169.


A
L
AC
E
EC
P
Fig.11



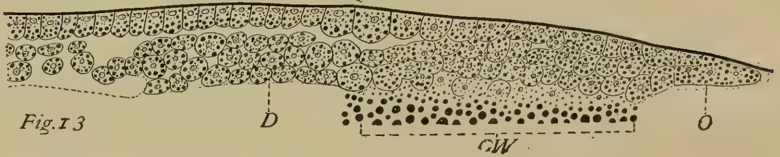


FIG. 11. A median longitudinal section of a blastoderm taken thirty-eight hours after fertilization, or three hours before laying. × 72.

FIG. 12. Enlarged anterior portion of the subgerminal cavity of the section represented in Fig. 11. × 169.

FIG. 13. Enlarged posterior portion of Fig. 11. × 169.

ever, these cells spread out and at the same time become flattened thus filling the intervening spaces and producing the characteristic gut-entoderm. At the stage represented in this figure, the entoderm in its forward growth has not yet reached the anterior limit of the subgerminal cavity, but its free edge ends about .35 mm. from this point (Fig. 11, *L*). The anterior part of the cavity not yet penetrated by the entoderm is occupied mainly by large yolk masses (Fig. 12). In the posterior part of this section the entoderm is directly continuous with the *mass* of cells (Fig. 13, *D*), which in turn is continuous with the inner edge of the germ-wall, and posterior to this wall is the region of overgrowth (*O*). In order to prove the origin and significance of this mass one must have recourse to experimental data. This form of evidence shows that the right and left halves of the dorsal lip of the blastopore grow toward each other and fuse in the median plane, that is, in the plane of the future longitudinal axis of the embryo. This movement of material from the lateral halves is not confined to the dorsal lip alone, but is participated in by the more lateral portions of the margin, that is, by the horns of the germ-wall. In the large majority of blastoderms, however, the right and left horns of the germ-wall do not turn in along the median line and fuse, but their free ends, upon meeting simply coalesce and grow out over the yolk[1] (see Fig. 6). It should also be borne in mind that as the horns are moving toward the median line, they are at the time being carried centrifugally by the expansion of the blastoderm. In this way the fused halves of the blastoporic lip are enclosed just anterior to the inner edge of the germ-wall, and hence the mass of cells, referred to above, is derived from the deeper portions of this enclosed lip. This is most apparent immediately after the ends of the horns have met, when the ectoderm is not yet differentiated from the underlying mass.

The movement of the two lateral halves of the posterior margin toward the median line and their simultaneous fusion must be regarded as a form of "concrescence"—the right and left halves of the dorsal lip representing the "homotypical" halves of the future embryo.[1]

---

[1] In the cases in which a "marginal notch" is present, and in the rare cases such as that described by Whitman ('83) the horns of the germ-wall must also turn in and fuse.

A reconstruction of the blastoderm represented in Figs. 11–13 is shown in Fig. 14. The over-growth (*O*) and both zones of the germ-wall (*Y* and *Z*) completely encircle the blastoderm. Just anterior to the inner edge of the posterior germ-wall is the mass of cells (*R*) which is thick in the center, but gradually thins out towards the sides.[2] Anteriorly it is continuous with the entoderm, into which it is differentiating as the latter grows forward through the subgerminal cavity. At this stage the entoderm (*E*) still ends with a free edge anteriorly, but its lateral margins are united with the yolk-sac entoderm at *S*. The entoderm does

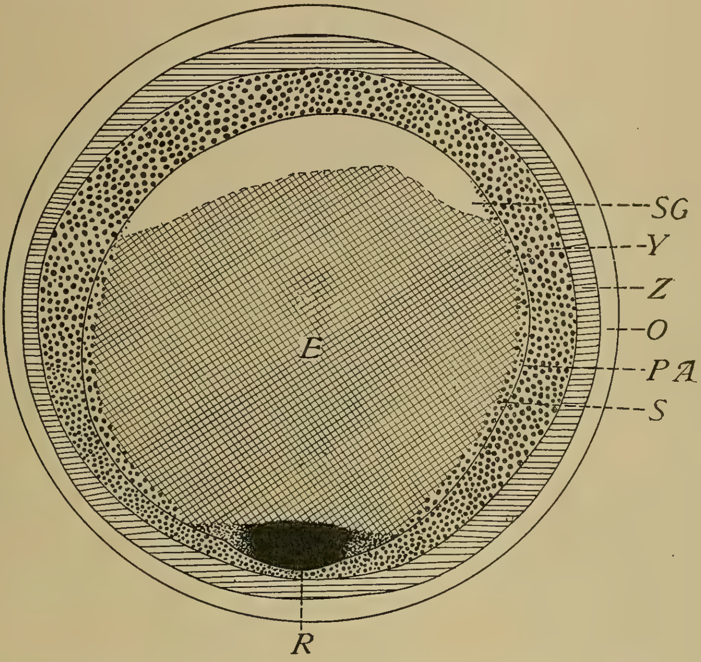


FIG. 14. A diagrammatic reconstruction of the blastoderm represented in Figs. 11–13. See Fig. 6 for the significance of the lettering. *R*, mass of cells, or the deeper cells of fused halves of the dorsal lip. × 27.2.

---

[1] This view does not differ essentially from that advanced for the chick by Rauber ('76), Whitman ('78 and '83) and others — a view hitherto not supported by experimental workers, mainly because they experimented upon stages which were too far advanced.

[2] This mass probably corresponds to what Koller ('81) called a "Sichel."

not reach the anterior limit of the subgerminal cavity until from two to four hours after laying, at which time the mass disappears.

Origin of the Primitive Streak.

The primitive streak in the pigeon's blastoderm becomes visible in surface views between the fourth and fifth hours of incubation. In sections, however, it can be detected as early as two hours previous to this time. Its first appearance in section is that of small protuberances of cells on the under surface of the ectoderm situated along the median line. In their longitudinal extension these swellings reach from the posterior edge of the *area pellucida* to a point lying about half way between this edge and the center of the blastoderm (Fig. 15, *ps*). Under high magnification (Fig. 17, *ps*) these swellings are seen to be groups of rapidly dividing cells, which at first are separated from the gut-entoderm, but upon further growth come in contact with it. At the stage represented in Fig. 17 the gut-entoderm is a single layer of flattened cells and is directly continuous with the yolk-sac entoderm (Figs. 15–17, *Y*). The latter is thicker just posterior to the primitive streak than in any other region of the yolk zone.

The evidence afforded by a study of gastrulation indicates the line along which one must look for an explanation of the origin of the primitive streak. During the progress of concrescence there are laid down along the sides of the future longitudinal axis of the embryo strips of primary ectoderm, which are fused along the median line. These strips were previously the superficial cells of the right and left halves of the dorsal lip of the blastopore. For four or five hours after the closing of the blastopore, this median strip cannot be distinguished from the adjoining ectoderm. It is not until the protuberances begin to make their appearance that any difference can be seen, and even then, the double structure of the median region is not evident. In fact, it is only when the primitive groove appears that this bilateral structure becomes clear.

In conclusion, I may say, that during the process of gastrulation only the gut-entoderm is involuted; the chorda and mesoderm arise from the primitive streak, which represents the fused

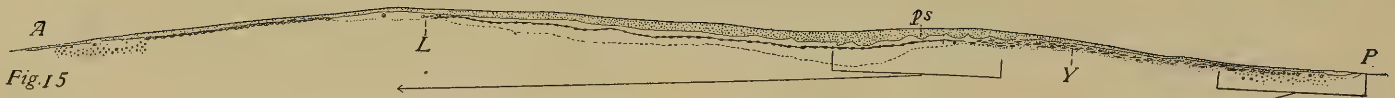


Fig.15

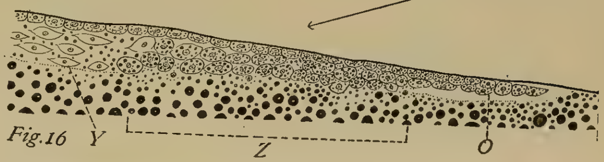


Fig.16

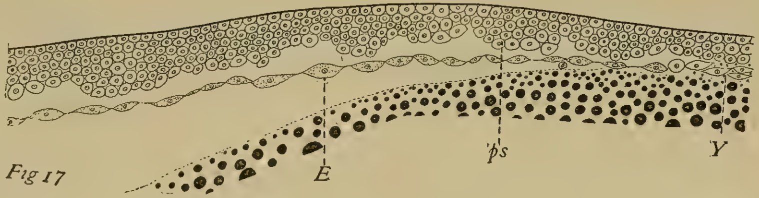


Fig 17

FIG. 15. A median longitudinal section of a blastoderm incubated four hours. It shows the appearance of the primitive streak as first seen in section. × 41.

FIG. 16. Enlarged portion of the posterior end of Fig. 15. × 169.

FIG. 17. Enlarged portion of the primitive streak region of Fig. 15. × 169.

halves of the dorsal lip of the blastopore. The main evidence in support of this view is derived from experimental data, which will be presented in a future paper.

It gives me pleasure here to acknowledge my indebtedness to Professor Whitman, under whose direction the work has been carried on, and also to Professor F. R. Lillie for help and criticism.

Hull Zoölogical Laboratory,
University of Chicago,
July 10, 1907.

Common Reference Letters used in the Figures.

A	Anterior end of the blastoderm.
AC	Archenteric cavity.
B	Blastopore.
E	Invaginated or gut-entoderm.
EC	Ectoderm.
F	Floor of the subgerminal cavity.
GW	Germ-wall.
L	Anterior limit of the gut-entoderm.
M	Yolk masses.
N	Periblastic nuclei.
O	Region of overgrowth.
P	Posterior end of the blastoderm.
R	Dorsal lip of the blastopore.
SC	Segmentation cavity.
SG	Subgerminal cavity.
V	Vitelline membrane.
Y	Yolk zone.
Z	Zone of junction.

LITERATURE CITED.

1. **Agassiz, L., and Whitman, C. O.**
'84 On the Development of Some Pelagic Fish Eggs. — Preliminary Notice. Proc. of the Amer. Acad. of Arts and Sci., Vol. XX.

2. **Assheton, R.**
'96 An Experimental Examination into the Growth of the Blastoderm of the Chick. Proc. Roy. Soc. London, Vol. LX.

3. **Blount, Mary.**
'07 The Early Development of the Pigeon's Egg, with especial reference to the Supernumerary Sperm Nuclei, the Periblast and the Germ-wall. — A Preliminary Paper. Biol. Bull., Vol. XIII.

4. **Duval, M.**
'84 De la Formation du Blastoderme Dans L'œuf D'oiseau. Annales Des Sci. Nat., 6 Serie, Vol. XVIII.

5. **Gasser, E.**
'82 Beitrage zur Kenntnis der Vogelkeimscheibe. Arch. f. Anat. u. Physiol.

6. **Harper, E. H.**
'04 The Fertilization and Early Development of the Pigeon's Egg. The American Journal of Anatomy, Vol. III.

7. **Hertwig, O.—Mark.**
'99 Text-book of Embryology.

8. **Koller, C.**
'81 Untersuchungen über die Blätterbildung im Hühnerei. Arch. f. Mik. Anat., Bd. XX.

9. **Kopsch, Fr.**
'02 Ueber die Bedeutung des Primitivstreifens beim Hühnerembryo. Leipzig.

10. **Nowack, K.**
'02 Neue Untersuchungen über die Bildung der beiden primären Keimblätter und die Enstelung des Primitivstreifens beim Hühnerembryo. Inaug. Diss. Berlin.

11. **Peebles, Florence.**
'98 Some Experiments on the Primitive Streak of the Chick. Archiv. f. Ent Meck. Organ., Band VII., Heft 2 u. 3.

12. **Waldeyer, W.**
'69 Bemerkungen über die Keimblätter u. den Primitivstreifen bei der Entwicklung des Hühnerembryo. Zeitschr. f. rationelle medicine.

13. **Whitman, C. O.**
'78 The Embryology of Clepsine. Quart. Jour. of Micro. Sci., Vol. 18.

14. **Whitman, C. O.**
'83 A Rare Form of the Blastoderm of the Chick. Quart. Jour. of Micro. Sci., Vol. 23.

15. **Zeigler, —.**
'02 Lehrbuch der Vergleichenden Entwickelungsgeschichte der niederen Wirbeltere.

THE EFFECTS OF SALTS AND SUGAR SOLUTIONS ON THE DEVELOPMENT OF THE FROG'S EGG.

T. H. MORGAN AND C. R. STOCKARD.

The purpose of these experiments was to determine more definitely what action takes place when eggs of the frog are treated with solutions containing both salts and sugar, as compared with control solutions containing only salt or sugar. We have also tried to compare the action of such sugars as cane sugar, that might possibly invert, with the action of simpler sugars, like glucose or lævulose. We have kept in mind the possibility that while the chief action of sugar is through its osmotic pressure, yet sugar may also act chemically on the living substance; or by forming new compounds with the inorganic salts may affect the results in this way.

Action of Lithium Chlorid and Sodium Chlorid Acting Alone.

The concentration of LiCl that will prevent the development from proceeding beyond the segmentation stages was previously determined for the egg of *Rana sylvatica* to be about 0.65 per cent. Since the salts obtained at different times may vary in the amount of absorbed water, it was necessary (for percentage solutions were used) to make a new determination as control for exact comparisons with the effects of the new salt when united in solution with sugars. The eggs were put into the solutions in the 2-cell stages in all cases. It was found that in LiCl 0.5 per cent. (the strength used in combination with sugar) the blastopore appeared and sometimes closed almost normally; usually it formed a ring around or below the equator of the egg. Lithium chlorid of this strength is, therefore, near the limit of inhibitory effects but below that limit. Some solutions of this strength were used with sugar solutions.

The upper limit of NaCl was not accurately determined in previous work on the frog[1] and the results are not in harmony with the present ones. In NaCl 0.5 per cent. development was nearly normal; in 1.0 per cent. only the 16- and 32-cell stages were reached; in 1.5 per cent. only the 16-cell stage, and in 2.0 per cent. only the 8-cell stage, and in the 2.5 per cent. only the 4- or 8-cell stage was reached, while in the 3.0 per cent. the eggs died without developing further. Similar results were obtained in another series of the same kind. In a third series of smaller range it was found that in a 0.5 per cent. solution the later cleavage stages were reached; in a 0.7 per cent. solution also only the late cleavage stages appeared; in a 0.9 per cent. solution the cleavage had not gone so far, while in a 1.0 per cent. solution only the 32- or 64-cell stages had been formed.

---

[1] Morgan, T. H., "Experiments with Frog's Egg," Biol. Bull., XI., 2, 1906.

The upper limit for NaCl lies, therefore, somewhere between 0.5 and 1.0 per cent. and not above 2.0 per cent. as previously stated. This difference in the results may possibly have been due to some impurities in the NaCl, not present in the salt used the previous year. The osmotic pressure for 0.5 per cent. is 3.55 atmospheres and for 1.0 it is 6.96. The latter is above that of LiCl 0.65 per cent., which is 6.16.

Action of Solutions Containing both Salts and Sugars.

Previous work on the frog's egg had indicated that when to a salt solution, too weak in itself to prevent development, a certain amount of sugar is added the development may be prevented; and a comparison of the osmotic pressures showed that in such a solution the pressure is higher than that when the salt alone produces the same results, but lower than that necessary for the sugar alone to produce the effect. Similar results have been obtained for the salt-water fish, *Fundulus*, where the outcome is even more striking owing to the fact that the eggs of this fish will not develop in a fresh water solution of salt and sugar that has an osmotic pressure lower than that of sea water in which they normally develop.[1] We have gone over these results with the frog in order to make sure that the effects were not due to the inversion of the cane sugar previously employed (that would increase its osmotic pressure) or that the effects were not due to an adulteration of the cane sugar with other sugars.

---

[1] Stockard, C. R., "The Influence of External Factors, Chemical and Physical, on the Development of Fundulus heteroclitus," *Jour. Exp. Zoöl.*, IV., 2, 1907.

In a ten per cent. solution of cane sugar the blastopore may develop, but in a thirteen per cent. solution only the later cleavage is reached. In a 5.5 per cent. solution of glucose the blastopore may develop in an abnormal way, while in a 6.0 per cent. solution only the late cleavage stage is reached. The results show that the same effect is produced by the same osmotic pressure of the two sugars.

A double solution of LiCl 0.5 per cent. plus glucose 0.5, 0.7, and 1.0 per cent. gave the following results:

LiCl 0.5 per cent. plus glucose 0.5 per cent., late segmentation.
LiCl 0.5 per cent. plus glucose 0.7 per cent., late segmentation.
LiCl 0.5 per cent. plus glucose 1.0 per cent., segmentation more abnormal.

The action of LiCl 0.4 per cent. plus glucose 1.0, 2.0 and 2.5 per cent. was as follows:

LiCl 0.4 per cent. plus glucose 1.0 per cent., very late segmentation: abnormal blastopore.
LiCl 0.4 per cent. plus glucose 2.0 per cent., late segmentation; not so far.
LiCl 0.4 per cent. plus glucose 2.5 per cent., late segmentation.

The results show that by adding amounts of glucose to a solution of LiCl, the development is stopped at a pressure higher than that for LiCl alone, but less than that for glucose alone.

Another similar experiment gave the same results. In a third experiment with LiCl 0.4 per cent. plus glucose 0.5, 1.0, 1.5 and 2.0 per cent. the results were nearly the same as is shown below.

LiCl 0.4 per cent. plus glucose 0.5 per cent., gastrulation normal, but delayed.
LiCl 0.4 per cent. plus glucose 1.0 per cent., barely gastrulating: abnormal.
LiCl 0.4 per cent. plus glucose 1.5 per cent., barely gastrulating: abnormal.
LiCl 0.4 per cent. plus glucose 2.0 per cent., late segmentation only.

The upper limit for this combination is about LiCl 0.4 per cent. plus glucose 2.0 per cent. with an osmotic pressure of 6.63, which is above LiCl .65 (= 6.16), but lower than glucose 6.0 (= 8.376).

The results with NaCl plus glucose were as follows: In a solution of 0.5 NaCl plus glucose 1.0, 1.5, 2.0 and 3.0 per cent. the late segmentation stages developed, but the yolk was injured. In another experiment the results were more decisive.

NaCl 0.5 per cent. plus glucose 1.0 per cent., circular blastopore above equator.
NaCl 0.5 per cent. plus glucose 1.5 per cent., late segmentation.
NaCl 0.5 per cent. plus glucose 2.0 per cent., dead in segmentation stages.
NaCl 0.5 per cent. plus glucose 3.0 per cent., not so late segmentation.

The upper limit for this combination lies therefore about NaCl 0.5 per cent. plus 3.0 per cent. glucose.

The Action of Sugar Solutions.

The experiments with sugar solutions were conducted to find if possible an explanation of the peculiar results which Stockard had obtained during the past summer by treating *Fundulus* eggs with solutions of sugars and sugar and salt mixtures. When *Fundulus* eggs were subjected to a solution containing LiCl or NH_4Cl and cane sugar the action of the salt was greatly augmented by the presence of the sugar even though the osmotic pressure of the mixture was lower than that of sea water. This indicated that the more marked action was not due to any increase in osmotic pressure that may have resulted from the addition of the sugar, but to some further or new chemical action.

Equal amounts of sugar were found to exert a more injurious effect on *Fundulus* eggs when in fresh water than when in sea water, although obviously the osmotic pressure of the latter was much the greater. This seemed possibly to indicate that the cane sugar in the fresh water solutions had become inverted, thus producing these pecular results. From a consideration of the experiments below it would seem more probable, however, that sugar exerted some chemical action on the compounds of the egg when in fresh water solutions rather than that inversion had taken place resulting only in an increase of pressure.

Frog eggs when in the four-cell stage were subjected to the following solutions of cane sugar: 6, 8, 9, 10, 11, 12, 12.5, 13, 15, 17 and 20 per cent. Although this is a series of fairly wide range it was found that the eggs were only slightly affected in the 6 per cent. solution, while they reached a late segmentation stage even in the 15 per cent. solution; the limit of effectiveness or fatal dose of sugar is thus seen not sharply indicated as in the case of many salts where a small fraction of a per cent. difference in the concentration of the solution gives at the critical points a marked difference in the effects on the eggs. The specific gravity of the sugar solutions was so high in most cases that the eggs would float in an indifferent position, the greater weight of the yolk pole not serving as it normally does to orient the egg in a definite manner. The sugar solution was freshly prepared before starting the experiments, and Heines' solution was used to test the sugar to further assure ourselves of its purity and uninverted condition.

The 6 per cent. cane sugar solution delayed the rate of development after about twenty hours, so that when forty-eight hours old the eggs were far behind the control; and although neural folds and other indications of the embryo were present the embryonic outline was usually shortened as an effect of the delayed blastopore closure. The 8 per cent. solution gave much more marked effects. After twenty-four hours abnormal gastrulæ were formed, though none became elongated or showed any indication of embryo formation. Such a condition is similiar to that described below for eggs in 4 and 5 per cent. solutions of glucose and lævulose; the pressures of the 5 per cent. solutions are, however, slightly more than that of the 8 per cent. cane sugar. After fifty hours all of the eggs in the 8 per cent. solution were dead. Cane sugar of 9 per cent. had much the same effect.

Solutions of 10, 11, 12 and 12.5 per cent. cane sugar gave rather uniform results. Development was delayed within ten hours or less and usually stopped before gastrulation had commenced. In the 10 per cent. solutions, however, some eggs formed very abnormal gastrulæ of a rather uniform type, the upper dark or micromere portion of the egg had sunken in the lower coarser cells suggesting somewhat in gross appearance an acorn held in its saucer-like burr. It is of interest to note that such a type of gastrula was also found in the 5 per cent. glucose and lævulose solutions which exert approximately the same pressure as the 10 per cent. cane sugar.

The 13 and 15 per cent. solutions act much the same, the weaker giving less marked effects than the stronger one. After ten hours the eggs were much delayed, the white area had not been encroached upon by the darker cells and was divided into only six or eight large blastomeres. The eggs were all much plasmolized and development after twenty-two hours in the solution had progressed only about as far as control eggs of nine or ten hours old. Late segmentation was reached, and the eggs died in this condition after forty-five hours.

The 17 per cent. sugar exerts a pressure of about 11.86 atmospheres above that of the normal medium in which these eggs live. The eggs were effected very readily under such conditions. After only six hours in the solution a few showed no cellular structure at all, while the others were far behind the control in their rate of development. All died in late segmentation stages.

The 20 per cent. cane sugar stopped the development of most eggs within six hours, the blastomeres seemed to have fused together. A very few eggs divided to about the sixth or seventh cleavage and then underwent cytolysis.

Lactose or milk sugar was tried but this substance dissolved so slowly that it was difficult to interpret the results, and since the maximum pressure of the solution was reached only after the eggs had developed much beyond the 4-cell stage, the effects are not readily compared with those resulting from the use of the other sugars.

Simple sugars, glucose and lævulose, which are the inversion products of the cane sugar molecule were tried in order to determine if possible whether solutions of these which were isotonic with a given cane sugar solution would give similar results. Approximate comparisons of these solutions may be made as follows: A 5 per cent. solution of glucose or lævulose exerts a pressure nearly the same as a 10 per cent. solution of cane sugar. It is not exactly the same since a molecule of cane sugar, $C_{12}H_{22}O_{11}$, is a little less than twice a molecule of glucose, $C_6H_{12}O_6$, and in addition to this it must also be borne in mind that equi-molecular solutions of glucose and cane sugar do not exert exactly the same osmotic pressures, although for general purposes the two are considered about equal.

Eggs when in the four-cell stage were placed in the following strengths of glucose, 2, 3, 3.5, 4, 5, 5.5, 5.8, 6, 6.5, 10 and 15 per cent. The 2 per cent. solution had a very weak action causing the development to proceed slower than usual. After forty nine hours in the solution many of the eggs were slightly abnormal.

The 3, 3.5 and 4 per cent. solutions retard development considerably within twenty hours, and those in the 4 per cent. solution show abnormal gastrulation with prominent yolk-hernias.

The eggs in the latter solution are also plasmolized. In all three solutions the eggs died after about fifty hours as abnormal gastrulæ.

Eggs in 5 per cent. glucose in one of the experiments, formed abnormal gastrulæ, which closely resembled those described above in the 10 per cent. cane sugar, but in other experiments only a few eggs attempted gastrulation, and the majority died while in late segmentation stages. In the 5.5, 5.8 and 6 per cent. solutions late segmentation was reached, but all eggs were badly plasmolized with the animal pole flattened. Eggs in a 6.5 per cent. solution died in much the same condition, though plasmolysis occurred earlier in this solution.

Eggs underwent only a few divisions after being subjected to the 10 per cent. glucose solution, the effect was much the same as that of the 20 per cent. cane sugar.

In the 15 per cent. glucose the eggs were readily killed, the blastomeres being ruptured after only one or two divisions, or within about one hour after being subjected to the solution. This serves to convey some idea of how quickly these high osmotic pressures produce an effect.

Lævulose solutions of 2, 3, 5, 5.5, 6, 6.5, 8 and 10 per cent, were used. The general effects of such solutions were almost identical with those described for the same percentage solutions of glucose, showing that the actions were in the main part due alone to their osmotic pressures, and not to any difference in chemical action which the sugars might have exerted. One would not expect the chemical action of these sugars to be marked even if it was at all perceptible.

A consideration of the responses of frog's eggs to sugars would seem to indicate that the more violent action on *Fundulus* eggs of fresh-water solutions of cane sugar when compared with sea-water solutions is not due to the sugar in the fresh water having become inverted. It will be recalled that *Fundulus* eggs are more susceptible to the same percentage solution of a salt in fresh water than in sea water. These facts together with the case before mentioned of the augmented effect produced when sugar is added to a weak solution of a salt in fresh water, even though the pressure of the solution is below that of sea water, go to show that the results with these eggs are not due so largely to osmotic pressure, but more to some chemical action which appears to take place between the constituents of the egg substance and sugars or salts when contained in fresh-water solutions. It may be that something present in the usual medium in which they develop, the sea water, prevents to some extent such an action, thus both salts and sugar act less violently when applied in sea-water solutions. The physiological condition of the egg may be weakened in fresh water as is indicated by its slightly retarded development in this medium, and under such circumstances they may be more susceptible to external injurious influences.

It is possible that new substances may be formed when sugar is added to salt solutions since some salts, *e. g.*, NaCl may form sodium-sugar compounds. Such compounds might be more or even less toxic than the original chemicals from which they resulted. These suggestions, which are at best speculative, serve to show how far we are from an understanding of the manner in which eggs respond to chemical stimuli, and indicate the importance of obtaining more complete data on the subject.

ZOÖLOGICAL LABORATORY,
COLUMBIA UNIVERSITY, May, 1907.

THE SEGMENTAL ORGAN OF PODARKE OBSCURA.

LOUISE HOYT GREGORY.

In 1892 Goodrich[1] described "A New organ in the Lycoridea." A ciliated organ found on the dorsal side of the body, paired in every segment except the first and last few. The nephridium was found to be a well-developed organ, opening into the body cavity by a ciliated nephrostome. The narrow and tortuous character of the nephridial tube made it impossible to conceive of its acting as a genital duct considering the large size of the eggs. A comparison of the dorsal ciliated organ with the genital funnels of the capitellids described by Eisig — however, suggested the idea that the dorsal ciliated organ might function as a genital funnel. Goodrich could discover no external pore, nor was he able to observe the discharge of the genital products. Yet he considered it possible that the external pore might be formed only at maturity.

In the spring of 1905, Professor Wilson suggested that I examine this question by the study of sexually mature individuals of *Nereis* and at the same time work out the method of the discharge of its sex products. *Heteronereis* material, found in abundance at Woods Hole, was collected in the summers of 1905 and 1906 but no definite result was obtained on this form. In the case of *Podarke obscura*, however, I had better success, and although the results conform in general to the work of Goodrich on other members of the family Hesionidæ, yet they may be sufficiently different in detail to warrant their description.

The material was collected in July and August, 1905, and in June and July, 1906. Mature females were fixed before the time of discharge (which takes place regularly between 7–9 p. m.),[2] during the period of discharge, and after the eggs had been passed out from the body. Immature specimens, collected in

---

[1] Goodrich, E. S., "On a New Organ in the Lycoridea and on the Nephridia in *Nereis diversicolæ*," *Quarterly Journal of Microscopical Anatomy*, 1892–3, vol. 34.

[2] Treadwell, A. L., "Cytogeny of *Podarke obscura*," *Journal of Morphology*, 1900–1, vol. 17.

June were fixed as well as males obtained throughout the season. Flemming's fluid and sublimate-acetic were found to be the best fixatives. The material was then sectioned and stained in iron hæmatoxylin.

I wish to thank Professor Wilson who suggested the work and under whose direction it has been completed. Thanks are also due Professor Treadwell and Dr. McGregor for their aid in collecting material.

The nephridia of *Podarke* are present in pairs in every segment of the body except the first few. A general cross-section of the body (as is seen in Fig. 1) shows a fairly typical annelid structure, *i. e.*, the outer layer of epithelial cells, a thin layer of circular muscles, and two pairs only of longitudinal muscles, a ventral and a dorsal pair. In such a section the nephridium is found following the dorsal surface of the ventral longitudinal muscle band. It is a simple tube with practically no convolutions, opening to the exterior laterally on the ventral surface, at outer limit of the ventral longitudinal muscles. (This opening is definitely shown in Fig. 4, the transverse section did not pass through the opening.) The nephridium extends along the upper surface of the muscle, then bends diagonally inward in the segment toward the median plane and forward toward the anterior dissepiment

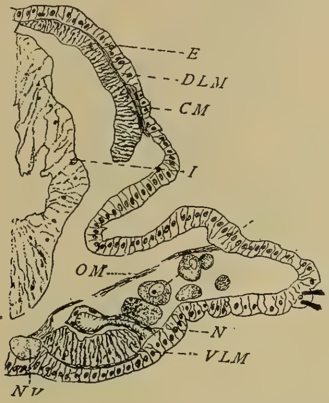


FIG. 1. × 115. A partly diagrammatic transverse section of body. *E*, ectoderm; *D.L.M*, dorsal longitudinal muscle; *C.M*, circular muscle; *I*, intestine; *O.M*, oblique muscle; *V.L.M*, ventral longitudinal muscle; *Nrv*, nerve; *N*, nephridium. (The inner end corresponds to the cavity seen in Figs. 2 and 3.)

where it unites with a large ciliated organ in the next anterior segment. (This union is shown in Figs. 2 and 3.) The walls of the nephridium are thin, its cells contain numerous excretory particles and its cavity is lined with fine cilia. The organ receives its blood supply from branches of a ventral longitudinal blood vessel. The position of the blood vessel is shown in the sagittal section, Fig. 2. The organ as a whole is simple in structure and may be regarded as a more or less degenerate condition of the nephridium as seen in *Hesione pantherina*, the nephrostome in the latter being replaced by the ciliated organ.

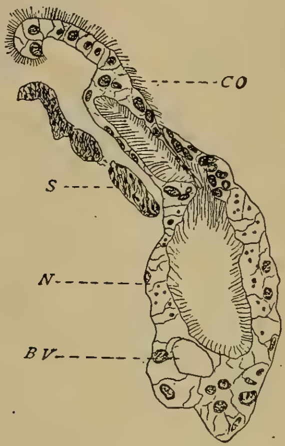


FIG. 2. × 500. A sagittal section of the segmental organ. Showing the relationship between the ciliated organ and nephridium. *C. O*, ciliated organ; *S*, septum; *N*, nephridium; *B. V*, blood vessel.

The ciliated organs of *Podarke*, like the nephridia, are paired in every segment behind the pharynx. The organ is a thin, flat, more or less triangular plate of ciliated cells, one layer in thickness, which stretches out into the body cavity anteriorly away from the dissepiment with the distal edge of the organ, or the base of the triangle turned toward the intestine and the apex of the triangular mass attached to the nephridium. The dorsal edge of the flat plate is rolled over toward the dissepiment. At the posterior edge it is often compressed and folded, but gradually broadens out into a large lip at the distal end. The ventral portion is only slightly rolled and in this case the turn is away from the dissepiment. This fold is present only at the distal anterior end of the ventral edge where it forms a short lip.

Immature specimens show the ciliated organ lying directly on the dissepiment, from the cells of the peritoneal covering of which it develops. In mature forms the organ does not lie against the dissepiment except at the point of union with the nephridium. In a sagittal section (Fig. 2) the organ is raised from the dissepiment. Although consisting of only one layer of cells, it may be folded in such a way as to give the appearance of more than one layer. This condition is seen in Fig. 2; if it were not compressed, it would have the appearance of the organ as seen in Fig. 3.

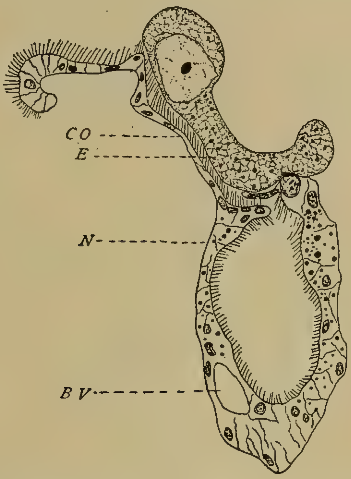


FIG. 3. × 500. A sagittal section of the segmental organ showing an egg passing along the ciliated surface. *C.O*, ciliated organ; *E*, ovarian egg; *N*, nephridial sac; *B. V*, blood vessel.

The ventral lip is not shown in either of the sagittal sections as they passed through the union of the nephridial tube and ciliated organ, whereas the ventral lip which is short and found only at the anterior edge (as has been stated), would appear in earlier sections in the series.

I have not found any indication of an internal termination of the nephridium other than the ciliated organ, and it seems evident that the latter forms an open cœlomic funnel. Its nature may best be considered after an examination of its relation to the discharge of the sex products.

In commencing the work, the material at first showed the nephridia to be so small that it seemed impossible that the large eggs could pass out through them. Goodrich's supposition that the eggs might pass to the outside through a break in the body seemed at first to be confirmed in some of my sections. I found cases where there appeared to be a definite rupture in the center of the ventral longitudinal muscles. This interpretation, however, was proved false, first by the fact that the eggs passing through the rupture were immature, ovarian eggs, whereas, in general, the eggs have been found to form their first polar spindle before leaving the body, and second, by the discovery of eggs passing along the ciliated organ and down into the nephridial cavity (Figs. 3 and 4). The walls of the nephridium are thin, but elastic and are capable of great expansion at the time of maturity. The inner end of the tube swells as the eggs become matured.

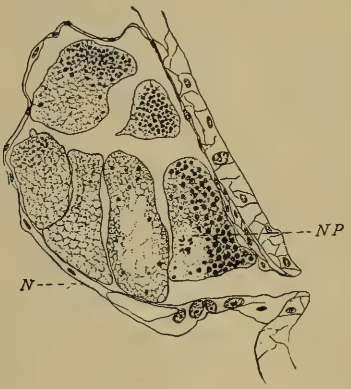


FIG. 4. × 500. A sagittal section of the nephridial sac showing the external opening at the lower end. *N*, nephridial sac; *N.P*, nuclear plate showing that the first maturation spindles are formed before the eggs are extruded. All of the eggs are in the same stage of development, the nuclear plates being visible in different sections.

(The beginning of this is shown in the nephridium in Fig. 1.) Finally the whole tube is distended and has the appearance of a large irregular sac filled with sexual products (Fig. 4). The eggs may pass into the nephridium before forming the polar spindles which are formed in that case, while the eggs are in the nephridial sac. This was probably the case in Fig. 4, or as has been observed by Treadwell,[1] the spindles may be formed after the eggs have passed out of the body; but, as a rule, the first spindle appears while the egg is free in the body cavity, before its passage into the nephridial cavity. In the male, the relations between the nephridium and ciliated organ is the same as that in the female, but the discharge of the spermatozoa was not observed.

Goodrich[2] in his summary of the most important facts resulting from his study of the Hesionidæ, says: *Hesione sicula* has a nephridium, which opens ventrally to the exterior, passes inwards and forwards, becomes considerably coiled, and finally ends just in front of the intersegmental region by a small, simple, funnel opening into the cœlome of the next segment. Connected with the lip of the nephrostome by a narrow strip of epithelium is a large crescentic genital funnel (ciliated organ), the ciliated surface of which is marked by deep grooves. Those of the middle region converge towards the loose extremity of the organ, where it is connected with the nephrostome and with the body wall. The exact mode of exit of the genital products is unknown.

In *Tyrrhena,* the nephridium is essentially the same but the genital funnel is smaller and more closely connected with the nephrostome.

In *Kefersteinia* and *Ophiodromus* it completely surrounds the inner extremity of the nephridium.

Finally in *Irma,* where the nephridium is no longer coiled, the large genital funnel surrounds and fuses completely with its inner end, forming a trumpet-shaped cœlomic funnel. The genital products, collected together by the action of its ciliated surface pass down into the nephridium and so to the exterior by the nephridiopores.

Podarke seems to be most similar to *Irma.* In both cases, the discharge of the genital products has been determined definitely to be by means of the ciliated organ and nephridium. In *Irma,* however, immature forms show the ciliated organ developing separately from the nephridium, the union taking place when the animal is sexually mature. In *Podarke* immature forms show the ciliated organ and nephridium developing in union with one another. The form of the ciliated organ is also quite different from that of *Irma.* There is no resemblance to a "trumpet-shaped organ," the organ being in the form of a flat, triangular mass, as has already been stated, and is united at one point only, not entirely surrounding the nephridium as in *Irma*, yet the rolling of the dorsal and ventral edges may indicate an incomplete funnel.

---

[1] Treadwell, A. L., "Cytogeny of *Podarke obscura,*" *Journal of Morphology,* 1900–1, Vol. 17.

[2] Goodrich, E. S., "On the Nephridia of the Polychæta," *Quarterly Journal of Morphology,* vol. 43, 1900.

Fage[1] has worked on these segmental organs in a number of forms in this family, and has found that in *Ophiodromus flexuosus*, *Oxydromus propinquus*, *Kefersteinia cirrata*, there is found a simple nephridium, slightly convoluted, opening to the exterior by a pore found in the region at the base of the parapodium, and into the body cavity by a straight nephrostome. At the moment of sexual maturity a ciliated organ formed from the peritoneum, is united with the nephridium and the sex products pass out through the compound organ. Goodrich did not observe this fact while working on the same forms.

In *Hesione pantherina*, the segmental organ is different, the excretory tube has many convolutions, the nephrostome is well developed possessing long cilia, the entire organ is much more highly modified than in other members of the family, and it closely resembles the excretory organ of the Lycoridea. At the time of sexual maturity, the organ undergoes no transformation. A ciliated organ homologous with that described in other forms of this group, is found near the nephrostome. It resembles the dorsal ciliated organ of *Nereis*. This organ develops at the same time as the nephridium and persists as an independent structure throughout the life of the individual. At its base is found the so-called phagocyte organ, containing in its meshes, granules comparable to the amœbocytes of the cœlome.

Thus we see that when the nephridium is highly developed and adapted more perfectly to its excretory function, it becomes more and more useless as a genital duct, and we find a special genital funnel appearing independent of the nephridium, and serving as a means of discharge for the sexual products. By this form, the family of Hesionidæ is closely connected with the family of Nereidæ.

---

[1] Fage, Louis, "Organes segmentaires des annelides Polychetes," *Annales des Sciences Naturelles*, Tome III., 1906.

Podarke obscura has been shown to possess a simple, uncoiled nephridial tube with a well differentiated ciliated organ developing at the same time and probably in union with the nephridium. No trace of a phagocyte organ was observed.

As a result of the investigations in this one family of the Polychætes, we may divide the Hesionidæ into three groups.

This classification is modeled somewhat after that made by Goodrich for the whole group of Polychæta. The nephridium has always an internal opening. Solenocytes have not been observed.

Group 1. — Forms in which the segmental organs consists of a nephridium highly differentiated, having a coiled tube and a well-developed nephrostome, and a distinct independent ciliated organ or genital funnel with an external opening through which the eggs are discharged. *Hesione pantherina.*

Group 2. — Forms in which the nephridium is a more simple tube opening into the cœlome by a small nephrostome. At the time of reproduction a ciliated organ is grafted on to the tube and affords a means of exit for the genital products. *Ophiodromus flexuosus, Oxydromus propinquus, Kefersteinia cirrata.*

Group 3. — Forms in which the nephridium is still more simple, having no coils. The nephrostome has been finally lost and its place taken by the ciliated organ or genital funnel, which develops at the same time, and probably in union with the nephridium. In this one group we may find forms illustrating the gradual loss of the nephrostome and the final fusion of the ciliated organ. *Hesione sicula, Tyrrhena, Irma, Podarke obscura.*

In this classification we have a gradual degeneration or modification of the nephridium from a condition where it performs its excretory function only, through a condition where it has become adapted temporarily to the secondary function of offering a means of sexual discharge to a condition finally where it has become so modified that from an early stage, if not throughout life, it functions as an excretory duct and a genital tube.

COLUMBIA UNIVERSITY,
April, 1907.

A PECULIAR LEGLESS SHEEP.

CHARLES R. STOCKARD.

A peculiar sport lately appeared in a flock of sheep in Nash County, N. C.[1] During the early part of February a black ewe gave birth to a lamb by a white ram ; the offspring was entirely devoid of all external indications of limbs and was black in color like its mother. This lamb has a perfect head and body with the usual long tail, and has been in a healthy condition since birth. It was fed on milk from a bottle for the first month or two, but is now able to eat grass and appears normal in size and other respects except for its apodal condition. The movements of this animal are limited; it can right itself if turned on its back and can twist its body about to some extent, but is entirely incapable of any progressive movements (Figs. 1 and 2, Pl. XIII.).

Photographs and descriptions of the lamb indicate that it is a sport or mutation rather than a merely deformed monster.

Another legless lamb almost identically like the first one was born in this same flock three months later. The two lambs had the same father (there being only one ram with the flock), but different mothers. This second lamb was a white male, and unfortunately was killed.

The parent ram is an old sheep, but no definite or authentic records of his previous offsprings can be obtained. It is a peculiar coincidence, however, that two of his young in so short a time should have shown this remarkable legless condition. Such a fact suggests, from the rather insufficient evidence, that this male has within his germ-cells a tendency to produce these lambs without legs.

The occurrence of the legless animals recalls the classical case of the ancon ram, a sheep with short crooked legs, that was born in Massachusetts about 1791. The ancon race was produced from this one ram by crossing at first with common sheep. The legless sheep here recorded seem to have carried such a variation a last step further, and it will be extremely interesting to know whether this character will tend to establish itself and produce a legless race.

---

[1] I have not up to this time been so fortunate as to personally observe these sheep, but the photographs and descriptions have been obtained from an entirely reliable source.

Zoölogical Laboratory,
Columbia University.

Explanation of Plate XIII.

Fig. 1. Shows a photograph of the apodal lamb with its ventral surface turned upward; the animal lying on its back.

Fig. 2. The lamb with its ventral surface toward the reader. Both photographs serve to show the entire absence of external limbs.

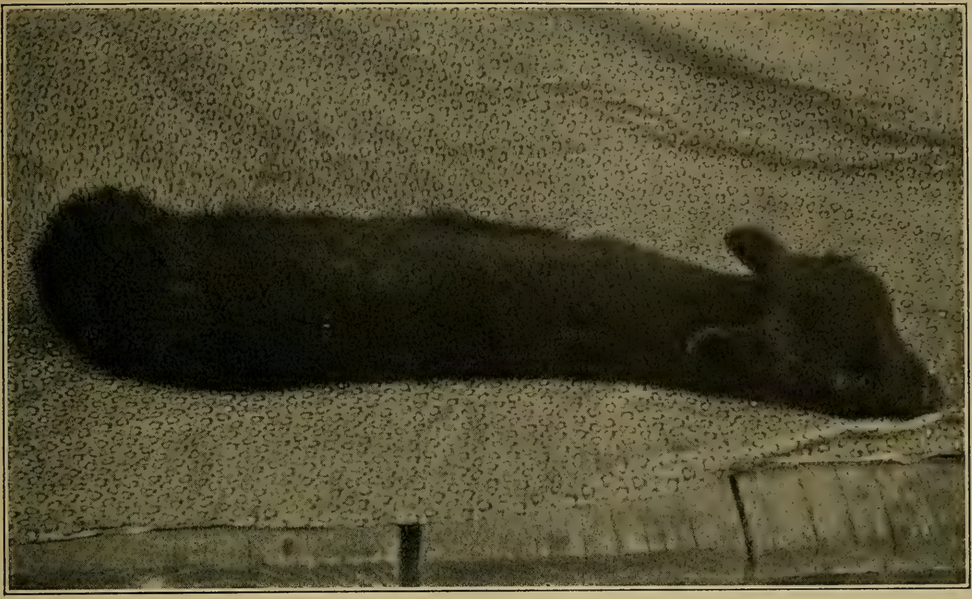


FIG. 1.

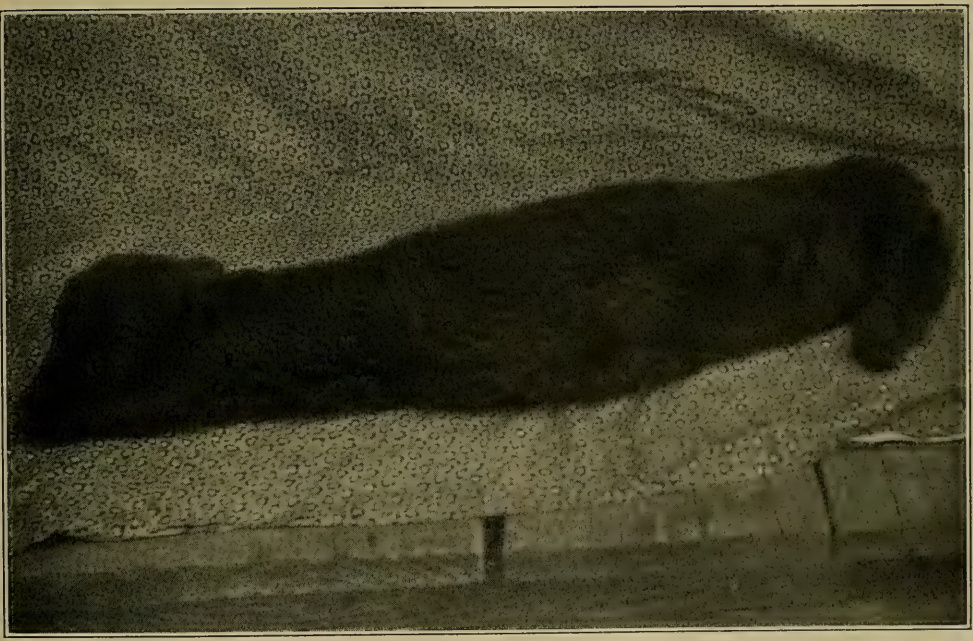


FIG. 2.

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

VOL. XIII   NOVEMBER, 1907   NO. 6

CONTENTS

		PAGE
D. D. WHITNEY.	*Artificial Removal of the Green Bodies of Hydra Viridis*	291
N. YATSU.	*A Note on the Adaptive Significance of the Sperm-head in Cerebratulus*	300
THOMAS H. MONTGOMERY, JR.	*On Parthenogenesis in Spiders*	302
S. J. HOLMES.	*Rhythmical Activity in Infusoria*	306
D. H. TENNENT.	*Further Studies on the Parthenogenetic Development of the Starfish Egg*	309
FERNANDUS PAYNE.	*The Reactions of the Blind Fish, Amblyopsis spelaeus, to Light*	317
S. W. WILLISTON.	*The Antennæ of Diptera; A Study in Phylogeny*	324
C. H. TURNER.	*Do Ants Form Practical Judgements?*	333
JULIAN MAST WOLFSOHN.	*The Causation of Maturation in the Eggs of Limpets by Chemical Means*	345

PUBLISHED MONTHLY BY THE

MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY

THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN	AGENT FOR GERMANY	AGENT FOR FRANCE
WILLIAM WESLEY & SON	R. FRIEDLÄNDER & SOHN	LIBRAIRIE ALBERT SCHULZ
28 Essex Street, Strand London, W. C.	*Berlin, N. W. Carlstrasse, 11*	*3 Place de la Sorbonne Paris, France*

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter,
under Act of Congress of July 16, 1894.

Editorial Staff.

E. G. CONKLIN—*The University of Pennsylvania.*
JACQUES LOEB—*The University of California.*
T. H. MORGAN—*Columbia University.*
W. M. WHEELER—*American Museum of Natural History, New York.*
C. O. WHITMAN—*The University of Chicago.*
E. B. WILSON—*Columbia University.*

Managing Editor.

FRANK R. LILLIE—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Holl, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa.

Vol. XIII. November, 1907. No. 6.

BIOLOGICAL BULLETIN

ARTIFICIAL REMOVAL OF THE GREEN BODIES OF HYDRA VIRIDIS.

D. D. WHITNEY.

Whether the green bodies in hydras are infecting algæ or products of their own metabolism has been long disputed. I have discovered a method by which they may be removed and the hydras continue to live nevertheless and to multiply.

Semper suggested that these green bodies in *Hydra viridis* might be algæ. A little later Brandt made a careful study of them and concluded that they are algæ which probably live parasitically in the endoderm cells of the hydra.

At the same time Lankester also studied these green bodies. He said: "It appears to me that an examination of the green colored corpuscles of *Hydra* demonstrates those corpuscles to be similar in nature to the chlorophyll bodies of green plants, and that there is no more reason to regard them as symbiotic algæ than there is to regard the green corpuscles in the leaf of a buttercup as such."

Sallitt examined the green bodies in several species of Protozoa and found them to be identical with the green bodies of *Hydra* and *Spongilla.* This uniformity of the green bodies in different animals and their similarity to the chloroplasts of plants led to the belief on the part of a few zoölogists that the green bodies in animals and plants are identical and probably have the same function in both kinds of organisms.

Beyerinck in 1890 isolated the green bodies from *Hydra viridis* and succeeded in making pure cultures of them in an artificial medium. This demonstrated that the green bodies were different from the chloroplasts of plants. He, moreover, identified them with the alga, *Chlorella vulgaris.*

Various theories have been advanced to account for the presence and function of this green alga in animal cells. Some workers have maintained that these cells assimilate the CO_2 which is given off by the animal cells, and then in their turn give off oxygen which is used in the life processes of the animal cells. Others have imagined that the algæ might manufacture products in the presence of sunlight which are passed out into the animal cells and used by them as a food.

Instead of a translocation of the algal reserves to the animal tissues Famintzin and Beyerinck have shown that the alga itself is liable to absorption and digestion by the host. Gamble and Keeble found that mature and immature *Convoluta roscoffensis* digest masses of their own green cells and that the animals obtain little if any food by the translocation of the reserves of its green cells.

They also found that the alga is not transmitted through the egg to the following generation as in the case of *Hydra viridis*, but that the young embryos of each generation are infected by the alga as soon as they leave the egg. Furthermore, "the relation between animal and green cells is a complex one, and cannot be described as symbiotic. The green cell once in the body of the animal probably never escapes; either it is digested or it dies when the animal dies."

In the winter of 1905–6 while keeping *Hydra viridis* in various chemical solutions in order to find some means of causing the development of the reproductive organs it was discovered that animals kept in a weak solution of glycerine lose their green color.

A series of experiments was carried on at that time and in the following winter and spring under the direction of Prof. T. H. Morgan and Prof. W. J. Gies. The following data will show the nature of the results obtained:

Experiment I.— February 19, 1906. Temperature 20°C. Several green hydras were put into a 1.25 per cent. solution of glycerine without food.

February 26. Only 3 were alive, and appeared white to the ordinary eye. These were placed in spring water without food.

March 3. The 3 hydras were beginning to become green in color at the oral end.

March 23. Hydras had normal green color.

Experiment II.— March 1. Temperature 20° C. Many green hydras put into a .5 per cent. solution of glycerine.

March 1. One hydra which appeared white to the eye, but which showed a single green patch of algæ in one tentacle under the lens, was isolated in spring water without food.

March 18. Green color had redeveloped gradually and was at this time identical with that of an ordinary hydra.

Experiment IV.— April 16. Temperature 20° C. Many green hydras put into a .5 per cent. solution of glycerine without food.

April 30. Four hydras which showed no green color under the lens were isolated in spring water without food.

May 7. The hydras showed no trace of green color under the lens. Experiment discontinued.

Experiment VIII. — May 5. Temperature 20° C. Many green hydras put into a .5 per cent. solution of glycerine. Fed every 72 hours with rotifers, *Hydatina senta.* Several formed buds in the glycerine solution.

May 24. Nine hydras which showed no trace of green color under the lens were isolated in a .25 per cent. solution of glycerine and the feeding continued. One individual had a bud attached which itself was budding. None of the others had buds.

May 26. One of the other 8 individuals was budding.

May 27. Three of the 8 individuals were budding.

May 29. Buds had become detached from two of the hydras. Experiment discontinued.

Experiment X. — March 12, 1907. Temperature 20° C. Many green hydras were put into a .5 per cent. solution of glycerine without food.

March 27. Began to add food every 24 hours.

April 7. Isolated 30 hydras which showed no trace of green color under the lens in small glasses containing spring water.

April 23. None had budded. Some had died and some were developing green color.

April 25. Fifteen hydras alive. Nine showed no trace of green color under lens and six showed green patches of algæ scattered in various parts of the body.

Lot A. April 25. Put three of the white hydras into a large balanced aquarium. Food added every 24 hours.

April 27. The three hydras were larger and each had one bud attached.

April 28. The buds had become detached from parent hydras.

April 30. The buds were larger than when detached. Each of the 3 parent individuals was budding again.

May 1. One of the parent hydras had two buds attached.

May 2. Nine individuals. Two of the parent hydras were budding. None showed a trace of green color under the lens.

May 5. Eleven individuals.

May 8. Twelve individuals, 5 of which were budding.

May 10. Sixteen individuals, 4 of which were budding, one had 2 buds. None showed a trace of green color under the lens.

May 27. Many white hydras on lighted side of aquarium.

Lot B. April 27. Put 6 white hydras into another large balanced aquarium which contained green hydras. The white hydras were not in very good condition; they were fed daily with rotifers.

May 1. Only two white hydras alive.

May 13. Several white hydras seen on walls of aquarium in the midst of the green ones. Some individuals were budding.

May 27. Many white hydras on the lighted side of aquarium.

Experiment XII.—April 4. Put one *Hydra fusca* together with many *Hydra viridis* into a .5 per cent. solution of glycerine without food.

April 14. Food added.

May 1. Twelve of the *Hydra viridis* had lost all green color. *Hydra fusca* was reddish orange in color. It had produced several buds.

May 8. Eight *Hydra fusca*, three of which were budded. Much larger and very different in color from the *Hydra viridis* that had lost their green color.

May 13. Thirteen *Hydra fusca*. Two individuals had 1 bud, 3 had 2 buds and 2 had 3 buds attached.

Experiment XIII. — April 14. Put several green hydras into a .5 per cent. glycerine solution. Food added daily.

May 10. Some hydras had no visible green color and were rather small in size.

Lot A. May 10. Put 5 of the white hydras into spring water without food.

May 20. All alive and white. Four were on the lighted side of the jar.

May 27. Five hydras alive. Two showed no green color but 3 had small green patches around the oral end.

Lot B. May 10. Put 6 of the white hydras into spring water without food.

May 20. All alive and white. Three were on lighted side of jar and 3 were on the bottom of the jar.

May 27. Four hydras alive. One showed no green color but 3 had small green patches around the oral end.

The action of the glycerine upon the green hydra is to cause the algal cells to leave the entoderm cells and to pass into the digestive cavity, from which they are expelled to the outside through the mouth when the hydra contracts. In many instances when hydras were examined which had been in the glycerine solution for a few days, masses of the green algæ could be seen in their digestive cavities. In one case the expulsion of the algæ was actually seen. In other cases no masses of the algæ were ever seen. However, the bottom of the glass upon which the animals were usually located always became more or less green within a small radius of each individual, showing that the algæ were expelled and sank to the bottom of the dish. The algæ never increased to any noticeable extent, but apparently died.

It seems evident from these experiments and observations that the alga in *Hydra viridis* does not play a very important rôle in the life processes. The animal is able to live many days in the glycerine solution without food while in the process of losing its algæ. In Experiments IV. and X. the hydras were without food for about 14 days, and in other experiments the animals have appeared to be in a normal condition, except smaller in size, when kept in glycerine solution without food for 21–30 days. Experiments IV. and XIII. also show that hydras that have lost all their green color can live at least for 7–17 days in spring water without food and at the end of this time be in a normal condition. Very likely they can live a much longer time than this without food.

The loss of the algæ does not seem to interfere with the process of bud formation in the slightest degree. Experiments VIII. and X. show that if the white hydras are fed sufficiently they will produce buds at a normal rate and in a normal manner.

It is a well-known fact that green hydras will live several weeks without food but of many hydras that I have kept 2–5 weeks in various experiments without food none ever produced buds, thus showing that the alga does not furnish food enough for bud formation. However, if sufficient food is given to either the white or green hydras buds soon appear.

The hydras from which all algæ have been extracted do not become reinfected with the algæ. In Experiment X. both lots of the white hydras were kept in large balanced aquaria in which there were hundreds of green hydras, but they remained white. Furthermore the white hydras were fed upon rotifers, the digestive tract of which was usually filled with *Euglena viridis.* It will be recalled that Sallitt believed that the green bodies of *Euglena* were identical with those of the green hydra. However, the colorless hydras are not infected by even this contaminated food supply.

The reappearance of the algæ in some of the supposed white hydras can be readily explained by the supposition that all of the algæ were not removed from the endoderm cells of the hydras before they were transferred to spring water. When the animals were placed again in their normal environments the alga began to grow and reproduce itself until the hydras became as green as normal ones.

The white hydras seems to respond to the stimulus of light in the same manner as the green hydras. In Experiment XIII., where the hydras were starved, some of them collected on the lighted side of the dish and the others remained upon the bottom. None were found on the least illuminated side of the dish.

In Experiment X., lot A, the white hydras were suspended in the large aquarium in a small dish. They did not leave the dish, but climbed upon its lighted side. The food, *Hydatina senta,* shows no reaction to light, so that it cannot be supposed that the hydras moved towards a food supply.

Some of the white hydras of lot B in the same experiment left the small dish, which was suspended in another large aquarium about three inches from the lighted side, made their way to the lighted side and there became attached. Those that remained in the small dish collected upon its lighted side.

Some of the early workers suggested that *Hydra viridis* might be a variety of *Hydra fusca* which had acquired the power of harboring green algæ in its cells. Greenwood and others found that the endoderm cells of green hydras contain brownish colored bodies similar to or identical with the same colored bodies in the cells of *Hydra fusca.* This brownish color is masked in *Hydra viridis* by the green color of the algæ.

Interesting as this suggestion is, the evidence from the colorless hydras is opposed to it. The *Hydra viridis* that has lost its green color in the glycerine solution is smaller in size, has shorter tentacles, produces fewer buds at any one time than *Hydra fusca.* It has a very faint tint of pink or brown color while *Hydra fusca* which has been in a glycerine solution 2–5 weeks has a reddish orange color.

Summary.

1. When *Hydra viridis* is kept in a .5–1.5 per cent. solution of glycerine it loses all its green color.

2. The green alga passes out of the endoderm cells into the digestive cavity from which it is expelled through the mouth to the outside when the hydra contracts.

3. The green alga does not continue to live outside in the glycerine solution.

4. The white hydras will live at least seventeen days without food.

5. The white hydras if fed can produce buds at the same rate and in the same manner as green hydras and have been kept alive for more than two months.

6. The white hydras do not show a trace of green color, under the lens, after seven weeks feeding upon contaminated food. These hydras were kept in spring water during the first 2–3 weeks of feeding and then placed in a large balanced aquaria which contained many hundreds of green hydras and much algæ. Thus showing that the white hydras were not reinfected under very favorable conditions.

7. The white hydra is positive heliotropic like the green hydra in moderate illumination.

8. The white hydra does not resemble *Hydra fusca* but, except in color, retains all the specific characters of *Hydra viridis* from which it is derived.

Zoölogical Laboratory,
Columbia University,
May 29, 1907.

LITERATURE.

Beyerinck, M. W.

'90 Kulturversuche mit Zoolchlorellen, Lichengonidien, u. anderen Algen. Botan. Zeit., Jahrg. XLVIII., pp. 725, 741, 757, 781.

Bouvier.

'93 La Chlorophylle animale et les Phénomènes de Symbiose entres les Algues vertes et les Animaux. Bull. Soc. philomath. de Paris, ser. 8, vol. 5, p. 72.

Brandt, K.

'81 Ueber das Zusammenleben von Algen u. Thieren. Verhandl. d. Phys. Gesell. Berlin, Nov. 11. Republished in Biol. Centralbl., Vol. I., p. 524.

'82 Ueber die morphologische u. physiologische Bedeutung des Chlorophyll bei Thieren. I. Arch. d. Anat. u. Phys., p. 125.

'83 Ueber die morphologische u. physiologische Bedeutung des Chlorophylls bei Thieren. II. Mittheil. Stat. Neapel., Vol. IV., p. 191.

'83 Ueber Symbiose von Algen u. Thieren. Arch. f. Anat. u. Phys., p. 448.

Engelmann.

'83 Ueber das thierische Chlorophyll. Pflüger's Archiv f. Physiol., Vol. XXXII., p. 80.

Enty, G.

'81 Ueber die Natur der Chlorophyllkorperchen der Thiere. Biol. Centralbl., Vol. I., p. 646.

'83 Das consortial verhaltniss von Algen u. Thieren. Biol. Centralbl., Vol. II., p. 451.

Famintzin.

'91 Beiträge zur Symbiose von Algen u. Thieren. Mém. de l'Acad. Imp. des Sciences, St. Petersburg, Vol. XXXVIII.

Gamble & Keeble.

'03 The Bionomics of Convoluta roscoffensis. Quart. Jour. Micros. Sci., Vol. XLVII., p. 363.

Geddes, P.

'79a Sur la Chlorophylle animale et sur la Physiologie des Planaires vertes. Archives de Zool. Exp. et Générale, ser. i., Vol. VIII., p. 51.

'79b Sur la Fonction de la Chlorophylle chez les Planaires vertes. Comptes Rendus, Vol. LXXXVII., p. 1095.

'81 The Yellow Cells of Radiolarians and Cœlenterates. Proc. Roy. Soc. Edin.

Greenwood, M.

'88 On Digestion of Hydra with some Observations on the Structure of the Endoderm. Jour. Phys., IX., p. 317.

Hadzi, J.

'06 Vorversuche zur Biologie von Hydra. Archiv. für Entw'm d. Org., XXII.

Kleinberg, N.

'72 Hydra.

Lankester, E. R.

'82a On the Chlorophyll Corpuscles and Amyloid Deposits of Spongilla and Hydra. Quart. Jour. Micros. Sci., XXII., p. 229.

'82b The Chlorophyll Corpuscles of Hydra. Nature, XXVII., p. 87.

MacMunn.

'90 Contributions to Animal Chromatology. Quart. Jour. Micros. Sci., XXX., p. 51.

Sallitt.

'84 The Chlorophyll Corpuscles of Some Infusoria. Quart. Jour. Micros. Sci., XXIV., n. s., p. 165.

Semper.

'79 Animal Life.

A NOTE ON THE ADAPTIVE SIGNIFICANCE OF THE SPERM-HEAD IN CEREBRATULUS.

N. YATSU.

While studying the fertilization processes in the living eggs of *Cerebratulus lacteus* at South Harpswell, Me., I was struck by the fact that it took the spermatozoa considerable time and not a little effort to bore through the thick membranes in order to reach the egg. From this I concluded that the long, slender and slightly curved head of the spermatozoön of *Cerebratulus lacteus* might have evolved in correlation with the thick egg-membrane characteristic of this species (Fig. 1, *A*).[1]

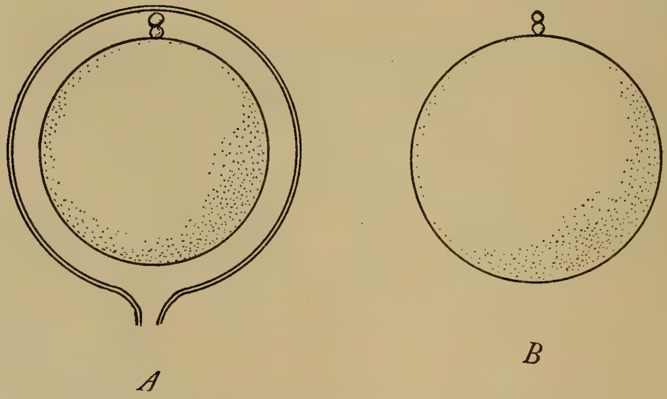


FIG. 1. Egg of *Cerebratulus lacteus*, (*A*), and of *C. marginatus* (*B*). × 220.

Since the study of sections was begun this conclusion has been strengthened. The spermatid has a round head. Later the anterior portion of the head is gradually drawn out into a slender beak, the head proper still remaining pear-shaped. At the last stage of this transformation the beak thickens and the head proper elongates. Thus the typical shape of the sperm-head is attained (Fig. 2, *A*). After entering the egg, the sperm-head repeats in reversed order the process just described, finally giving rise to a round sperm-nucleus, which does not differ much from that of the spermatid.

---

[1] The membrane is made up of two layers. By accident spermatozoa sometimes find their way into the space between them.

When informed by Professor E. B. Wilson that the egg of *Cerebratulus marginatus* has no membrane (Fig. 1, *B*), I thought that if the above conclusion were true, this species must have a spermatozoön with a blunt head. At Naples in the spring of 1906, I found that this expectation was fulfilled. Instead of the slender pointed head found in *Cerebratulus lacteus* (10.6 μ), the Neapolitan form (*C. marginatus*) has a spermatozoön with a blunt head (5.4 μ) terminating in a knob as shown in Fig. 2, *B*. The length of the tail is nearly the same in both forms.

The difference in size of the sperm-heads might be interpreted as due to the number of chromosomes contained in them. In fact *C. lacteus* has 18 or 19 chromosomes in the reduced number, while *C. marginatus* has 16 according to Coe.[1] Yet the difference in shape of the sperm-head between two such closely allied forms as these is difficult to explain without taking into consideration a special adaptation for boring thick membranes (cf. Pflüger and Smith, '83[2]). This, I think, is an actual instance to support the general belief that the diversity in the shape of sperm-head has evolved in response to the mechanical needs for penetrating the egg.

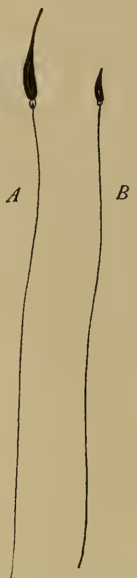


FIG. 2. Spermatozoön of *Cerebratulus lacteus* (*A*), and of *C. marginatus* (*B*). × 1133.

ZOÖLOGICAL LABORATORY,
COLUMBIA UNIVERSITY,
NEW YORK, June 8, 1907.

---

[1] Coe, W. R., '99, "The Maturation and Fertilization of the Egg of *Cerebratulus*." *Zool. Jahrb. Abth. Anat. u. Ont.*, 12.

[2] Pflüger, E., und Smith, W. H., '83, "Untersuchungen über Bastardierung der anuren Batracier und die Principien der Zeugung." *Pflüger's Archiv*, 32.

ON PARTHENOGENESIS IN SPIDERS.[1]

THOS. H. MONTGOMERY, JR.

The only references known to me upon the question of the occurrence of parthenogenesis in araneads are the following: Blackwall (1845) took young females of *Tegenaria domestica*, *T. civilis*, *Agelena labyrinthica*, *Ciniflo atrox*, *Drassus sericeus*, *Theridion quadripunctatum* and *Segestria senoculata* and kept "most of these individuals . . . in captivity from one to three years after they had completed their moulting and attained maturity; yet three only, an *Agalena labyrinthica*, a *Tegenaria domestica*, and a *Tegenaria civilis*, produced eggs, and they proved to be sterile, though several of the others, to which adult males were subsequently introduced, laid prolific eggs after coition." Blanchard (1857) also reached the same conclusion that eggs laid by unimpregnated females prove sterile. Then Balbiani (1873) adopted this conclusion, though his own observations were not decisive: "Having imprisoned during a whole year several females of *Tegenaria domestica*, I have noted that the first batches were composed exclusively of fecund eggs, while the subsequent batches contained always a variable number of sterile eggs, of which the quantity increased with the batch, so that they ended in not containing a single egg able to develop. But it is evident that if these females had been gifted with the faculty of reproducing without the concourse of the male, all the successive batches should have been equally fecund."

On the other hand, Campbell (1883) kept a female of *Tegenaria guyonii* in captivity a whole year, during which she underwent two moults; then she laid eggs from which young hatched. And Damin (1893) imprisoned a female *Filistata testacea* Latr. from the spring of 1891 until the spring of 1893; she moulted twice in the summer of 1891 and once in the spring of 1892, then made a cocoon from which young spiders emerged. He notes the extreme rarity of the males of this species, and asks: 'Does not this absence of the male indeed indirectly cause the, parthenogenesis of *Filistata*?" It may be remarked that *Artemia salina* is an instance of this kind. A paper by Holmberg (1878) has been inaccessible to me.

---

[1] Contributions from the Zoölogical Laboratory of the University of Texas, no. 86.

It is generally held that the females of spiders are not ready for coition until they have passed their final moult, and that not until then does the copulatory plate, the epigynum, become fully developed. However, Bertkau (1885) has shown that *Atypus piceus* oviposits several years in succession, and a moult occurs (with change of the seminal receptacles) after the first years of egg-laying; he remarks that the same is probably true also of *Gnaphosa lucifuga*. This would show that spiders may undergo moults after they are fully mature, and indeed it is very likely that when the female lives several years, and this is known to be the case in a number of species, she undergoes a moult each year after reaching maturity, for moulting is a necessary integral part of the excretory process. Then I (1903) have described the case of a *Lycosa bilineata* (Emerton) (*L. ocreata pulchra* Montg.) that copulated successfully on June 3, and on the following July 12 moulted. During the present year I caught a *Filistata* with a cocoon containing young; she moulted on July 2 and again on August 28. These instances indicate that spiders may be sexually mature before their final moults.

But one can be sure that a female is immature when her epigynum is still a small, smooth plate, and that when she is in such a condition she cannot be impregnated. And during the course of earlier observations on the mating habits I have noted that males avoid females that are not mature. Accordingly, females of Entelogynæ found with their epigyna small and imperfect may be considered virginal.

During the spring of this year the common *Lycosa relucens* Montg. was found in large numbers in the early part of March, males and females running over the ground in a wood at Austin, Texas. At that time very few mature individuals of either sex were discovered, the greater number being one or two moults removed from the mature condition. When they become full grown they are rarely found running upon the ground in the daylight, but then usually remain hidden under leaves and stones. Twenty-two females were secured, some on the third and the others on the ninth of March, and kept isolated in glass cases, with fairly rich feeding, until June 12. All of these when caught showed the epigyna small and imperfect and this fact, in connection with the observation that few of the males at that time of the year were mature, made it certain that all the females were virginal. All underwent moults during captivity, one moulted twice, the others only once; after moulting all but four made cocoons containing eggs. Eleven of the spiders made one cocoon each, seven made two cocoons each, and one made three cocoons. Of the total of twenty-eight cocoons, eleven were destroyed by the spiders shortly after their construction, the mothers eating the eggs, and most of these cocoons were very imperfect; while the remaining seventeen were removed from the mothers immediately after their completion to save them from such possible demolition, handled as gently as possible, and kept in separate bottles to test their fecundity. But not one of the eggs in any of these seventeen cocoons hatched, nor even reached the stage of the early blastoderm; one batch of eggs was fixed at the age of twenty-four hours, but on examination showed no cleavage nuclei near the surface, so they had certainly not reached the sixteen-cell stage. All these eggs were shrivelled and dry.

Therefore virginal females of *Lycosa relucens* form cocoons with eggs in them, but these eggs do not develop. And such females show always more or less imperfect construction of the cocoons and the tendency to eat the eggs; in mature individuals of *Lycosa* that I have bred in captivity, the eggs always developed, and the mother rarely ate the eggs.

My observations corroborate those of Blackwall and Blanchard, and in the species watched by us normal parthenogenesis seems not to occur; on the other hand, there are the two positive cases of its occurrence mentioned by Campbell and Damin. Certainly parthenogenesis is exceptional in spiders.

LITERATURE.

Balbiani, G.

1873 Mémoires sur le Devéloppement des Aranéides. Bibl. L'Ecole des Hautes Études, 7.

Bertkau, P.

1885 Ueber den Saisondimorphismus und einige andere Lebenserscheinungen bei Spinnen. Zool. Anz., 8.

Blackwall, J.

1845 Report on some Recent Researches into the Structure, Functions and Oeconomy of the Araneida, etc. 14th Rep. Brit. Ass. Adv. Sci.

Blanchard.

1857 Observations relatives à la génération des Arachnides. C. R. Acad. Sci. Paris, 44.

Campbell, F. M.

1883 On a probable Case of Parthenogenesis in the House-spider (Tegenaria Guyonii). Journ. Linn. Soc. London, 16.

Damin, N.

1893 Ueber Parthenogenesis bei Spinnen. Verh. zool.-bot. Ges. Wien, 43.

Holmberg, E. L.

1878 Caso de partenogenesis en una Arana. Periodico zoologico, 2.

Montgomery, T. H., Jr.

1903 Studies on the Habits of Spiders, particularly those of the Mating Period. Proc. Acad. Nat. Sci. Philadelphia.

RHYTHMICAL ACTIVITY IN INFUSORIA.

S. J. HOLMES.

While much in the behavior of the infusoria comes under the head of direct responses to external stimuli, there is, in many forms, an extraordinary amount of activity which cannot be traced to any outside cause. In addition to those forms which keep up a continuous swimming with never-flagging energy, there are several infusorians which perform movements of a more or less regular rhythm. These are analogous to such rhythmical movements as the beating of the heart of higher animals, or the rhythmical pulsations of the swimming of a jelly-fish. They are more automatic than the latter, and are, perhaps, more closely comparable to the regular pulsations, which, under certain conditions, are performed by some species of jelly-fish after removal of the nerve ring and marginal sense organs.

More or less regular and apparently spontaneous movements have been noted in a few species (*Stentor*, *Vorticella*) by various writers, but the subject has received scarcely more than a passing mention. My attention was called to this feature of the behavior of certain infusoria in some studies recently made on the behavior of *Loxophyllum meleagris*, and I was led to look for similar phenomena in other forms. Observations were made on the following species :

Loxophyllum meleagris. — *Loxophyllum* commonly moves about on some solid object by extending the body, gliding forward a short distance, then swimming backward, turning toward the oral side and then going forward again. The changes in the direction of movement are not due to any obstacles encountered ; they occur in much the same way when there are no objects in its course. The organism frequently keeps up this kind of movement for a long time in very nearly the same locality. The body is always narrowed in swimming forward, and always widened in swimming backward, thus showing a constant correlation of the contractile activities with the direction of the ciliary beat. If the body is cut in two, the pieces will undergo the same rhythmical back-and-forth movements. Even very small pieces, less than one sixteenth of the body, show the same regular rhythm and the same correlation of ciliary and contractile activity.

Dileptus gigas. — *Dileptus gigas* commonly adheres to the surface of some solid object and waves its long proboscis-like anterior extremity or neck about in an anti-clockwise direction. The surface of the body is quite sticky, as is shown by the fact that it adheres readily to any object brought in contact with it. The slender extremity in its movement about in a circle executes many twists and curls in more or less irregular ways. These movements may be very vigorous or they may be very slow, but they scarcely ever entirely cease. The slender neck is very extensile and may be elongated to three or more times its length when in a contracted state.

Dileptus often executes short forward and backward movements at tolerably regular intervals. During its movement forward the body elongates, and while gliding backward it widens, showing the same correlation of contractility with the direction of the beat of the cilia that occurs in *Loxophyllum meleagris.* The backward and forward excursions vary exceedingly in length. Frequently they are exceedingly short. Even when the organism remains attached in one place the body undergoes more or less regular elongations and contractions while waving about the anterior extremity. There is a rhythm here much as in the preceding species occurring quite independently of external stimulation. The posterior third of the body when severed from the rest still undergoes elongations and contractions, although in a somewhat lessened degree. In larger pieces the rhythm of movement is more manifest.

Lachrymaria olor. — This interesting species resembles *Dileptus gigas* in its general behavior as well as its external form. Its long flexible neck is kept continually waving about, but the extensions and contractions of its body do not occur so regularly as in the preceding species.

Vorticella. — *Vorticella* frequently shows quite regular rhythmic contractions without an apparent external cause. The peristome with its membranellæ is folded in and the stalk contracts into a spiral form. In a short time the spiral straightens out, the peristome expands, and another contraction soon follows. Hodge and Acking found that the interval between successive contractions in *Vorticella* varied greatly. In one individual kept for a long time under continuous observation contractions occurred at one time about once in four seconds, at another once in eight seconds, and at various other intervals in different times. Sometimes there was no rhythmic contraction at all. The stimulus to the rhythmic contraction of the stalk apparently comes from the body, for the stalks which I have isolated showed no independent movements.

Stentor. — *Stentor cæruleus* when attached and extended sways about slowly in a circle. The swaying is a very regular movement and is not due to any evident external stimulus. It is a result of the contraction of the body instead of the action of cilia, as the stalk is bent successively in different directions. There is also a rhythmic movement executed by *Stentor roeselii* during the construction of its tube. This species after attaching itself alternately contracts and extends its body in a more or less regular manner while it is secreting the gelatinous substance of which its tube is formed. The swaying movements are not so pronounced as in the preceding species.

It is a noteworthy fact that rhythmical activities occur in those species which are either attached like *Stentor* and *Vorticella*, or which like *Loxophyllum*, *Dileptus* and *Lachrymaria* frequently remain for a long time near one spot. These forms do not have to wait for something to turn up, but are actively seeking for new stimulations, their rhythmical movements bringing them in a measure the advantages which in forms like *Paramœcium* are secured by almost continuous swimming.

Biological Laboratory,
University of Wisconsin.

FURTHER STUDIES ON THE PARTHENOGENETIC DEVELOPMENT OF THE STARFISH EGG.

D. H. TENNENT.

The results of investigations described in an earlier paper[1] led me to the view that possibly a conjugation of egg and sperm chromosomes, similar to that apparently occurring in starfish eggs that had been treated with CO_2 and subsequently fertilized, might be found to occur in normally fertilized eggs.

As I suggested in the paper mentioned, it would be necessary, in order to settle the question raised, to reëxamine the normal fertilization and cleavage stages or to make a study of the formation of the germ cells in the starfish.

This paper deals with observations made in accordance with this plan and with some further observations made on starfish eggs. developing as a result of treatment with CO_2. The material for the investigation was obtained while I was occupying a room at the Marine Biological Laboratory, Woods Hole, during a portion of the summer of 1906.

I found, soon after beginning a study of fertilized starfish eggs. that the equatorial plate of the first cleavage spindle contained, with variations which I shall mention later, in eggs from some individuals 18 chromosomes, and in eggs from other individuals 36 chomosomes. I have as yet been unable to correlate this difference in the number of chromosomes with the common starfishes of the Woods Hole region, *Asterias forbesii* and *Asterias vulgaris*,[2] although it is probable that such a relationship will be established.

The study of the fertilized eggs proved puzzling, and it was not until I had made an investigation of the spermatogenesis of *Asterias vulgaris* and a reëxamination of eggs developing parthenogenetically after treatment with CO_2 that I was able to find a solution for the problem under consideration.

---

[1] "Studies on the Development of the Starfish Egg," D. H. Tennent and M. J. Hogue, *Journal of Experimental Zoölogy*, Vol. III. (1906).

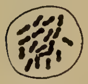
[2] Clark, "The Echinoderms of the Woods Hole Region," Bull. U. S. F. C., Vol. XXII. (1902), pp. 553-554.

Inasmuch as the basis of my interpretation lies in facts observed during the study of the male germ cells, I shall first present a brief account of these observations.

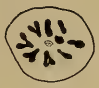
THE SPERMATOGENESIS OF ASTERIAS VULGARIS.

In well-preserved stronger Flemming material stained in iron-hæmatoxylin the spermatogonia show 18 chromosomes, these all having a slightly constricted or dumb-bell form (Fig. 1). The chromosomes are either straight or slightly bent.

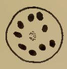
The chromosomes of the primary spermatocytes are nine in number and have at first a distinct dumb-bell form. A precocious longitudinal splitting soon gives them a V or looped form which may be seen in horizontal sections of the equatorial plate (Fig. 2).



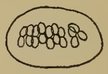
1



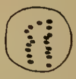
2



3



4



5

FIG. 1. Equatorial plate of spermatogonial mitosis.
FIG. 2. Spermatocyte of the 1st order.
FIG. 3. Spermatocyte of the 2d order. Polar view.
FIG. 4. Spermatocyte of the 2d order. Metaphase.
FIG. 5. Second spermatocyte division.

The secondary spermatocytes contain nine chromosomes (Figs. 3 and 4). In the second maturation mitosis these appear to be divided transversely (Fig. 5), giving nine as the reduced germ-cell number.

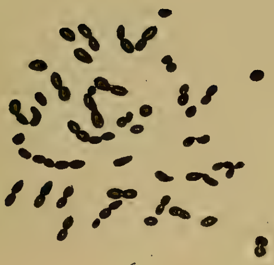
STUDIES ON EGGS.

After noting the difference in the somatic number of chromosomes in the different lots of eggs sectioned, it became evident that it was desirable to have a set of material in which the eggs from one individual had been treated in three different ways, namely, one set fertilized with sperm, another set treated with CO_2 and a third set treated with CO_2 and subsequently fertilized. I succeeded in obtaining one lot of material of this nature. I shall record my observations in the order of the above statement.

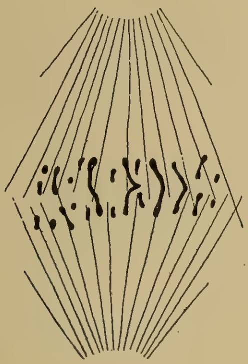
(*a*) *Observations on Fertilized Eggs.*

In a successful preparation, the section being sufficiently thick to show the greater number of the chromosomes of the equatorial plate of the first segmentation spindle in their entirety (Fig. 6), the chromosomes are seen to be of a dumb-bell form, some straight, some slightly bent, and lying with their long axis placed transversely to the spindle fibers. Their number, as may be seen from the figure, would lead one to suspect 36 as the somatic number, but owing to the fact that some of the chromosomes are cut, this may not be stated with certainty.

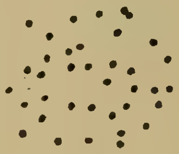
In an especially fortunate section passing symmetrically through the long axis of the spindle, it is seen that the chromosomes have been split longitudinally and drawn out as somewhat slender rods. In drawing this figure I have shown only the chromosomes and parts of chromosomes lying within a short focal range, inasmuch as it would have complicated the figure so greatly as to make it unintelligible had the chromosomes lying toward the opposite side of the spindle been added. In this section again, the full number of chromosomes could not be counted with certainty.



6



7



8

FIG. 6. Equatorial plate 1st segmentation fertilized egg. Polar view.

FIG. 7. First segmentation fertilized egg. Anaphase.

FIG. 8. First segmentation fertilized egg. Section through chromosomes as they are drawn out in anaphase.

Finally, in a section passing transversely through the spindle of an egg in the same stage of division as that from which Fig. 7 was drawn, it is shown conclusively that the somatic number of chromosomes in this lot of fertilized eggs is 36.

(*b*) *Observations on Eggs Treated with CO_2.*

Due precautions were of course taken to avoid chance fertilization. The control showed freedom from segmenting eggs.

In the sections of these eggs it was even more difficult than in the fertilized eggs to determine accurately the number of chromosomes. Sections thick enough to contain all of the chromosomes were unintelligible. Thinner sections were likewise of little value. Fig. 9 shows all of the chromatic material contained in the equatorial plate as demonstrated in two sections of this egg. The impossibility of stating with any reasonable degree of accuracy the number of chromosomes involved is evident to any observer. Nor is the situation appreciably relieved by the examination of longitudinal sections of the spindle in later anaphase.

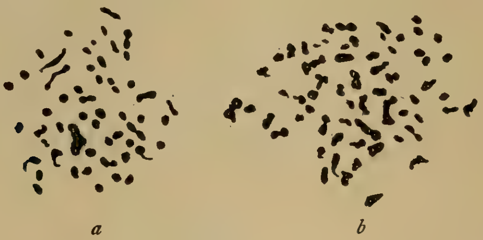


FIG. 9. *a–b*. Sections through same equatorial plate CO_2 egg.

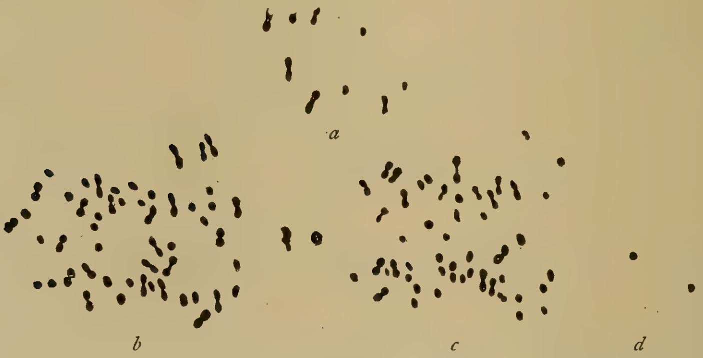


FIG. 10. *a–d*. Four longitudinal sections through 1st segmentation spindle, late anaphase. CO_2 egg. Spindle fibers omitted.

In such sections as those shown in Fig. 10, *a–d*, a cursory examination would lead the observer to believe that the number of chromosomes is fully as great as that in the fertilized eggs. This view might be supported by the fact that many of the chromosomes show a form similar to that possessed by those of the fertilized eggs.

Closer examination of the position and arrangement of the chromosomes in these sections reveals the fact that the bodies have been sectioned. The imaginary superposition of one figure upon the other lends credence to such an idea.

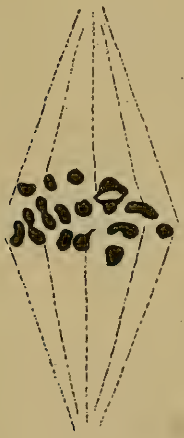
For these reasons I have been unable to determine the number of chromosomes by actual count. The number I believe to be 18, a statement for which I shall give my reasons later.

(*c*) *Eggs Treated with CO_2 and Subsequently Fertilized.*

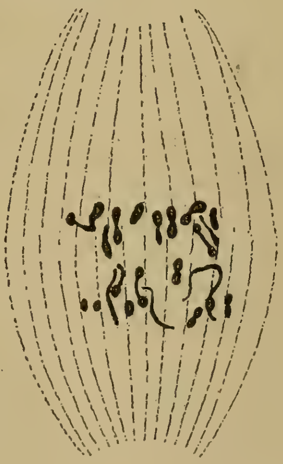
Sections of these eggs agree with figures that I have already published. The eggs were fertilized and underwent segmentation, the CO_2 simply retarding the rate of development.

Observations on Other Starfish Eggs Treated with CO_2.

I succeeded in obtaining one lot of eggs which developed after treatment with CO_2, that contain in all cases only 9 chromosomes. I was unable to obtain a ripe male at the time and so can give no facts as to the fertilization of these eggs.



11



12

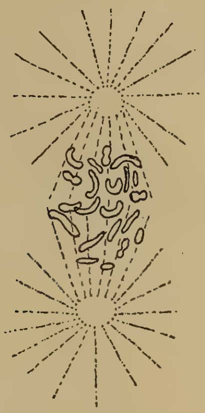
Fig. 11. First segmentation CO_2 egg. Early anaphase.

Fig. 12. First segmentation CO_2 egg. Later anaphase.

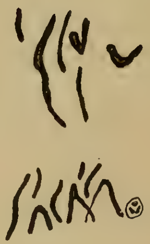
The individual from which I obtained the eggs I identified as *Asterias forbesii* although it will be noted that the number of chromosomes agrees with the germ-cell number in my spermatogenesis material of *Asterias vulgaris.*

In these eggs, as in the other CO_2 eggs described, the form of the chromosomes is irregular but owing to the smaller number may be counted readily. The equatorial plate, the daughter plates, etc., all show the same number, — *i. e.*, nine (Figs. 11 and 12).

Fig. 13 shows the extremely irregular form assumed by the chromatic material in anaphase and explains the reasons for the complexity exhibited by sections such as those from which Fig. 10 was drawn.



13



14

FIG. 13. First segmentation CO_2 egg. Anaphase.

FIG. 14. First segmentation CO_2 egg. Late anaphase. One chromosomal vesicle.

As shown in Fig. 13 this chromatic material at this stage is in the form of greatly twisted threads. A single section may cut the thread in several places.

In later anaphase these threads are drawn out, passing through a variety of changes and at last are embodied in chromosomal vesicles which unite to form the daughter nucleus (Fig. 14).

Clearly then, this egg with its oöcyte number of 9 chromosomes does not exhibit the phenomenon of "autoregulation."

As I have already stated, owing to my inability to make an accurate count, I have not been able to show this to be true in the case of the egg whose reduced number of chromosomes is 18. That the behavior of both eggs is probably similar will be granted by most readers.

General Considerations.

These observations show conclusively that in the fertilized egg there is no conjugation of maternal and paternal chromosomes as individuals, at the time when I thought that such a union might take place.

The facts that I have given, namely, that the reduced number of chromosomes in the male germ cell of one form is 9, that the reduced number of chromosomes in one egg is 18, and that the number in fertilized eggs is 18 and 36 is sufficient proof.

After the examination of many lots of fertilized eggs I became convinced that 18 and 36 were not constant as somatic numbers. Small variations, such as differences of one or two, might be laid to error in counting. A constant greater variation that I have found hardly seems due to the same cause.

A possible interpretation of such a greater variation is of interest.

In one lot of eggs the number 27 seems constant. In this lot I have never been able to count as many as 36 chromosomes.

Such a number, (27), is readily explained on the supposition that an egg containing 18 chromosomes has been fertilized by a spermatozoan containing 9, or that an egg with 9 has been fertilized by a spermatozoan containing 18. The result in either case would be a somatic number of 27.

Now, accepting the interpretation of synapsis as the conjugation of homologous maternal and paternal chromosomes, we shall have at the conclusion of synapsis a reduced number of 18. That is, nine pairs or nine bivalant chromosomes and nine univalents which had been unable to find mates.

Such eggs, if they were fertilized by a spermatozoan containing 18 chromosomes should give rise to individuals with a somatic number of 36, or, uniting with a spermatozoan with 9 chromosomes should retain a somatic number of 27.

If the theory of the individuality of the chromosomes is correct and the interpretation of synapsis well founded, experiments in hybridization with favorable forms ought to prove the truth of such an explanation. The starfish, owing to our inability to raise adults from the egg and to the extremely small size of its chromosomes, does not seem promising for such an investigation.

SUMMARY.

1. The reduced number of chromosomes in the male germ cells of *Asterias vulgaris* is 9.
2. Fertilized starfish eggs contain as a somatic number 18 and 36 chromosomes, the difference possibly to be correlated with *Asterias vulgaris* and *Asterias forbesii.*
3. Eggs caused to develop parthenogenetically show one half the somatic number of chromosomes.
4. No conjugation of individual chromosomes takes place in fertilized eggs immediately before the first segmentation.
5. A possibly hybrid form contains 27 chromosomes.

BRYN MAWR COLLEGE,
July, 1907.

All of the figures are from camera drawings made with aid of Zeiss No. 12 compensating ocular and 2 mm. apochromatic objective. Some of the sketches were subsequently doubled in diameter by means of a drawing camera. These have been reduced one half in reproduction. The others are reproduced as drawn.

THE REACTIONS OF THE BLIND FISH, AMBLYOPSIS SPELÆUS, TO LIGHT.[1]

FERNANDUS PAYNE.

Introduction.

In the Woods Hole Biological Lectures ('99) Dr. Eigenmann gave the results of some experiments to determine the reaction of *Amblyopsis* to light. He recorded that:

1. *Amblyopsis* seeks the dark regardless of the direction of the rays.
2. An individual coming from a dark chamber into a lighted one shows signs of uneasiness.
3. A light ray thrown on fishes from a mirror causes uneasiness in from one to five seconds.
4. Bright sunlight causes the fishes to swim uneasily.
5. A lighted match held above an aquarium, which had been in the dark, caused two fishes in one instance to dart to the bottom. In another case it produced a very general and active movement among forty individuals.
6. Different colors do not cause different reactions.
7. In an open pool, the fishes remained under rocks during the bright part of the day.

It is the purpose of the present paper to repeat some of his experiments, but in a different way; to add others, to give data in full of how the fishes react and to determine why they react. The special interest in the problem lies in the fact that we are dealing with a blind animal, whose remote ancestors possessed well-developed eyes.

Part of the work was done at the Indiana University cave farm at Mitchell, Indiana; the remainder at the university. All the material used was caught in the caves at Mitchell.

These fishes are very sensitive to mechanical stimuli and with this in mind every possible precaution has been used to eliminate them. At Mitchell the cave was used as a laboratory and the aquarium placed on the solid earth, so no better nor more natural conditions could have been found. At the university, the experiments were made in a basement dark-room with black walls, where the temperature remained practically constant. The aquarium was set on a stone pedestal.

---

[1] Contributions from the Zoölogical Laboratory of Indiana University, No. 89.

Only very simple experiments were made and conclusions have not been reached until after an examination of more than one series of fishes, although the data of only one series will be given.

Apparatus.

My apparatus consisted of an aquarium, a light screen, a heat screen and a lamp. The aquarium was 18 inches long, 15 inches high and 12 inches wide. The light screen was made of heavy cardboard and was placed between the end of the aquarium and the heat screen, so as to shut out all light except that entering from one end of the aquarium. A small aquarium was used for a heat screen. Its sides were of clear glass, parallel and placed 3 inches apart. This screen was placed 2¼ inches from the end of the aquarium. The water used in both the aquarium and heat screen was filtered. Two lamps were used, one an acetylene lamp of one hundred candle power, and the other an arc lamp of eight hundred candle power.

Experiments and Observations.

My experiments at Mitchell were made with the hundred candle power lamp. First, the lamp was placed 32 inches from the end of the aquarium and with it in this position the whole aquarium was lighted. As the aquarium was 18 inches in length there was considerable difference in the intensity of the light at the two ends. With ten fishes, ranging from 2½ to 4 inches in length in the aquarium, counts were taken once a minute for thirty minutes, and at the end of this time the counts showed 163 fishes in the end of less, and 137 in the end of greater intensity. Before making this observation and with the aquarium lighted just enough to enable me to see the fishes as white objects, I took counts to see whether they showed any preference for one end of the aquarium over the other. Thirty-five counts gave me 176 fishes in one end and 174 in the other. So the large number of fishes in the end of greater intensity could not have been due to a preference for that end.

With the light in the same position and with half of the aquarium darkened by means of a light screen, counts were again taken as before. First one half of the aquarium and then the other was made dark. Sometimes these counts showed more fishes in the dark ; at others more in the light. Under natural conditions the fishes swim very slowly. During these observations their movements were much faster, thus indicating that they are photodynamic. All subsequent observations, whether the light was of a low or high intensity, brought out this fact very distinctly.

On account of the shape of the lamp, it could not be brought closer than 32 inches and still illuminate the whole aquarium. I therefore made a small aquarium, 7 inches long, 5 inches high and 4 inches wide, and suspended it within the larger. In this case the heat screen was removed as there was always three or four inches of water between the fishes and the lamp. With the lamp only nine inches from the end of the small aquarium and with half of the aquarium darkened in various ways, I took a large number of counts. These observations were made on three series of fishes. The results were again conflicting. In the majority of cases a larger, sometimes a much larger number of fishes were seen in the dark, but sometimes a larger number were observed in the light. The fishes seem to be disturbed more with the light at this distance than when it is 32 inches from the end of the aquarium.

At the university an 800 candle power arc lamp was used. The heat and light screens were again placed in position and during the observations the entire aquarium, except the end where the light entered, was covered with heavy black cloth. The fishes were transferred from the cave to the aquarium in a closed vessel and hence were not exposed to the light. They were left in the aquarium, at least twenty-four hours before observations were made, so they could become accustomed to the new conditions. Also after taking one series of counts they were left several hours before making other observations. With the lamp 16 inches from the end of the aquarium, counts were taken every minute for thirty minutes and with ten fishes in the aquarium. First the right half was darkened. These counts gave me 84 fishes in the light to 216 in the dark. I then lighted the whole aquarium to see whether they would seek the end away from the light, *i. e.*, the end of less intensity. These counts gave me 85 fishes in the end of greater and 215 in the end of less intensity. Here is a difference of 130, while with the one hundred candle power lamp 32 inches from the end of the aquarium, there was a difference of only 26.

With the aquarium just sufficiently light for me to see the fishes, I took counts to see whether they remained at the surface or near the bottom. These counts showed 101 fishes in the upper half and 199 in the lower. This indicates that they are positively geotropic. To determine whether their positive geotropism would be overcome by their negative heliotropism, I darkened first the lower and then the upper half of the aquarium. With the lower half dark, the counts showed 56 fishes in the light to 244 in the dark and with the upper half dark 121 in the light to 179 in the dark. While their geotropic reaction is partly overcome by their negative heliotropism, it is not wholly so.

To determine whether the direction of the rays of light plays any part in the movements of the fishes, the light was placed 14 inches above the surface of the water. As no convenient heat screen was at hand, a clear glass which fitted snugly against all sides of the aquarium, was lowered 2½ inches beneath the surface. Thus there was always 2½ inches of water between the fishes and the light. With the lamp in this position, I darkened first one end and then the other and took counts as before. Thirty counts with the left end dark showed 68 fishes in the light and 232 in the dark, and with the right end dark 64 in the light and 236 in the dark. At the end of each count, I shifted the light screen to the opposite end, and each time the fishes within two or three minutes changed to the dark end again. This immediate change, with the shifting of the light, proves conclusively that the light is the only factor which caused them to seek the dark. These counts confirm the conclusion of Eigenmann that they seek the dark regardless of the direction of the rays.

With the light coming from above, we get a larger percentage of fishes in the dark than when the light strikes them from the side. It is possible that with the light overhead, the brain and spinal cord are affected directly on account of the transparency of the tissue above them.

For comparison, I caught a number of small specimens ranging from 15 to 25 mm. in length, placed 10 of them in the aquarium and made observations as I had done with the adults. In these fishes, the eye was plainly visible as a small black spot beneath the skin, while in the adults there is no external indication of an eye. With the light at the end of the aquarium and the whole aquarium lighted, the thirty counts showed 115 fishes in the end of greater and 185 in the end of lesser intensity, as compared with 85 to 215 in the case of the adults. With the left half dark there were 60 in the light to 240 in the dark, as compared with 98 to 202 in the adults. With the right half dark, 48 in the light to 252 in the dark, against 84 to 216 adults. The light was then placed above the aquarium as before and counts taken. When the left end was dark there were 28 fishes in the light to 272 in the dark and with the right half dark there were 25 in the light to 275 in the dark, as compared with 68 to 232 and 64 to 236 in the case of the adults. Here again we have a larger percentage of fishes in the dark when the light is above, and, further, these young fishes seem to be more sensitive to the light than the adults. What is the reason for this? Thinking that the eye might play some part in this difference I removed the eyes from 10 young. Seven of these recovered in good condition. Of these I took five, all of which were about an inch in length, and 52 hours after the operation made the first observation.

With the light at the end and the left half dark, thirty counts gave me 19 fishes in the light to 131 in the dark, and with the right half dark, 22 in the light to 128 in the dark, as compared with 48 to 252 and 60 to 240 in the case of the young with eyes. These observations show that there is practically no difference in the reactions of the young with eyes and those without eyes. Hence the eyes play no part in the reaction.

In making these experiments the counts often varied considerably in the same series even though the external conditions, so far as I was able to determine, were exactly the same. However, this is to be expected, since the fish is a highly complex organism and its internal mechanism is not the same at any two times.

To determine whether the skin is equally sensitive on all parts of the body, I used a light focused to a point by means of a Zeiss *a* * objective. With the aquarium dark, I focused this light on various parts of the body. Sometimes they reacted and sometimes they did not, but they reacted as often with the light focused on the tail as on the head, and *vice versa*. Later I placed some fishes in a dark corner of a room and with a mirror threw sunbeams on various parts of the body. In nearly every case I got a definite reaction. Sometimes the fishes turned around and swam in the opposite direction and sometimes darted forward. Further, they reacted as often when the light was thrown on the tail as on the head. Judging from these experiments they are equally sensitive on all parts of the body. However, this is what we might expect since all parts of the skin are exposed to like conditions. Parker ('05) concludes that the tail of Ammocœtes is most sensitive to light, but he accounts for this by the fact that Ammocœtes burrows head foremost into the sand.

Eigenmann ('99) states: "Two examples [of blind fishes] kept in a pail in my cellar were quietly floating, but when a lighted match was held above them, the fishes at once darted to the bottom and sides of the pail." This is not a common reaction. I have tried the lighted match again and again and also have flashed the one hundred candle power lamp above them, and in no case did they dart to the sides or bottom immediately. In fact, they did not react immediately when the eight hundred candle power lamp was flashed on them. With the fishes in their native habitat I have made a number of observations with the one hundred candle power lamp by flashing it upon them as they lay perfectly quiet in the water, and in each case it was from 10 to 30 seconds before any movement took place. In nearly all cases the movement was either to one side or straight ahead and not toward the bottom. My results are more in accord with his observation on forty individuals when a lighted match "produced a very general and active movement among all individuals."

In the same paper Eigenmann records the action of a colony of Amblyopsis in an open pool. "During the bright part of the day, the fishes always remain under the rocks at the bottom. In the morning and evening and at night they could be seen swimming about in various parts of the pool." At Mitchell, near the entrance of one of the caves, is a small pool, the bottom of which is covered with rocks. I found two fishes in this pool. They were probably washed there during times of high water, as the water runs from one cave to the other at such times. Later, I put two more fishes into the pool and as I was making daily trips to the cave I often noticed them swimming about near the surface. The pool was not in the direct sunlight but the sun reached it, in patches, between twelve and one o'clock, and I took a number of observations at this time. I watched the pool for 15 minutes at a time and out of 13 observations made on 13 different days was able to see from one to three fishes ten times out of the thirteen. Sometimes they came out only to go immediately back under the rocks, but they often remained at the surface from five to ten minutes. Apparently this seems to conflict with my former experiments, but such is not the case, because under no condition did the fishes remain in the dark all the time. I do not mean to say that in the pool the fishes remain in the light more than in the dark, but that they do come out at times even in the brightest part of the day.

CONCLUSIONS.

1. *Amblyopsis* is negatively phototropic.

2. The young are more sensitive to light than the adults.

3. The young deprived of eyes are as sensitive as those with eyes. Hence the eyes play no part in their reactions.

4. They seek the dark regardless of the direction of the rays.

5. When stimulated with a light focused to a point they seem to be equally sensitive on all parts of the body.

6. They are positively geotropic.

7. They are photodynamic.

8. These fishes are sensitive to light of low intensity and this sensitiveness increases as the intensity of the light increases.

THE ANTENNÆ OF DIPTERA; A STUDY IN PHYLOGENY.

S. W. WILLISTON,
Chicago, Ill.

No classification of the order Diptera is, on the whole, satisfactory. Four or five more or less elaborate schemes have been proposed by various writers in the past, but none has received general endorsement by students of the order. Scarcely any two writers agree as to the relationships of the larger part of the families, nor as to the value of many of their distinguishing characters. And this unsatisfactory condition is doubtless largely due to our failure to differentiate between homoplastic or convergent resemblances and genetic characters. As in all other large groups of animals there have been many phyletic lines of descent, many parallel adaptations to like environments, and these adaptive characters, here as elsewhere, have been, too often, used as fundamental classificational characters. Of past writers Osten Sacken was, I believe, most appreciative of such accidental resemblances; but even he often mistook adaptive for hereditary characters I am convinced.

The attainment of like characters by evolution by no means necessarily implies common ancestry. One would not think of uniting all flies having two-jointed palpi in one group, nor all those having a club-shaped abdomen in one suborder. But there are many other characters, less conspicuous ones, which have been used for such purposes, whose origins have been due to adaptations; and it will be long before we have thoroughly learned to distinguish them. New characters acquired in different phyla are seldom, perhaps never, exactly alike, though there may often exist the most curious resemblances, due doubtless to similar determining causes, or to orthogenesis, if there be such a thing. To paleontology we are indebted for the formulation, at least, of the apparent law that evolution is irreversible — that organs once functionally lost are never regained. There may be exceptions to this rule, but, so far, the history of past life seems to teach that there are not. A fly with two-jointed palpi, for instance, could not have been ancestral to one with four joints in these organs.

Of all the organs of diptera, the antennæ, it seems to me, have received less critical comparative study than any others, and I believe that there is a fertile field here for fruitful phylogenetic studies. The differentiation between the nematocerous and brachycerous flies was, for a long while, based almost exclusively upon the structure of these organs; until it was conclusively shown that, by themselves, they have little classificational value. The antennæ, for instance, of *Bibio* are so nearly identical with those of *Xylophagus* that they might be interchanged without affecting generic characters, even as the wings and palpi of the phorids might be interchanged with some of the scatopsines without affecting generic characters. It was doubtless because of this primary division long ago by Latreille and Macquart into "many-jointed" and "three-jointed" antennæ that a misconception still exists among many as to the real structure of these organs. There are very few three-jointed antennæ among diptera, and even these will usually show, under high magnification, vestiges of additional joints. The great majority of existing diptera have five or six joints in their antennæ, and this majority includes the whole of the Cyclorrhapha, with but few exceptions. The fallacy has been in considering the antennal style or "arista" as an outgrowth or addition to the real antenna, whereas it is of course merely the specialized and more or less attenuated distal (?) part of the flagellum, showing all stages of attenuation and abbreviation; and it is yet to be shown that the arista is quite homologous in all diptera.

In the following table I have condensed the results of considerable observation and research on the structure of the antennæ in the different families of flies. It is of course impossible for one to examine critically all the genera of diptera, and the published data are yet, in many cases, inexact, and this inexactness is especially apparent when it comes to the detection of vestigial or minute joints. I have found under critical examination not a few instances of minute joints which have been neglected by systematists in general. Such a study as the present one must take into account all vestiges and aborted organs if one would arrive at precise results. The Phoridæ, for instance, have been shown by Brues and others to possess two scape joints, though but one has usually been ascribed to them. And this will probably be found to be true of all the other so-called two-jointed antennæ, such as those of certain cyrtids, empidids and dolichopodids, under close examination. I have arranged the families in the following list not quite in the supposed order of their relationships, in order to bring out more clearly the antennal structure. I have also for the few families showing archaic forms given the extremes in parenthesis, with the "normal" or usual numbers in the regular column.

Tipulidæ (6–39)	12–16	Acanthomeridæ	10
Cecidomyidæ (6–36)	12–16	Tabanidæ	6–10
Psychodidæ	12–16	Leptinæ	3–8
Mycetophilidæ	12–16	Nemestrinidæ	5–6
Pachyneurinæ	12–16	Mydaidæ	4–5
Rhyphidæ	12–16	Apioceridæ	3–5
Dixidæ	15	Asilidæ	3–5
Culicidæ	14–15	Therevidæ	3–5
Blepharoceridæ	9–15	Scenopinidæ	3
Chiromomidæ	6–15	Bombyliidæ	3–5
Orphnephilidæ	11–12	Dolichopodidæ	4–5
Bibioninæ	8–12	Empididæ	3–5
Scatopsinæ	9–10	Lonchopteridæ	6
Simuliidæ	10	Phoridæ	6
Xylophaginæ (13–30)	9–10	Cyclorrhapha	5–6
Stratiomyidæ	7–10		

In this list we are at once struck with the predominance of five groups having the maximum normal number of sixteen, fifteen, ten, six and five. And I venture to suggest that these five groups represent, in the main, long since divergent phyla of diptera. Not invariably of course, because coincidences may and often do occur in the different lines of descent. There are quite a number, it is seen, having the maximum number of fifteen. Possibly these may represent one common branch from the sixteen-jointed antennæ, possibly several. But, in none of these groups, unless it be the fifteen jointed, and of course the primitive Tipulidæ and

Cecidomyidæ, do I believe that we shall often, if ever, find vestigial joints additional to the maximum — simply for the reason that the loss of additional joints has been so far back in geological history that vestiges have wholly disappeared.

If the law of irreversibility in evolution be true, then it is apparent that no fly has regained the use of a joint of the antennæ once functionally lost. The question at once becomes important: What was the original number of antennal joints in the Diptera? If we could only be assured of the origin of the order from the main insect stem, we might, perhaps, answer this question with satisfaction. But, since we cannot we are forced to depend upon the internal evidence presented by the diptera themselves. May we assume that this primitive number was sixteen, the number so conspicuous in the table? Or was it thirty-nine (or more) a number known in a single species of diptera? I have assumed that the evolution of the dipterous antenna has been by the reduction of the number of segments, and never by accretion. And I believe that this assumption is justified, though the matter is perhaps open to debate. There are very few forms of diptera known possessing more than sixteen antennal joints. Some species of *Pachyrhina* and of the nearly allied *Nephrotoma* among the Tipulidæ have nineteen joints in the male, fifteen or sixteen in the female. The genus *Ctedonia*, of the same family, from Chile, has twenty-two or twenty-four joints in the flagellum of the females of two species, fifteen in that of a third. . . . The very closely related *Cerozodia*, from Australia and New Zealand, with two species, known only in the males, has thirty-two and thirty-seven flagellar joints respectively, the largest number hitherto discovered in any dipteron. As Osten Sacken truly said: "The close affinity between *Cerozodia* and *Ctedonia* affords a new instance of the curious relationships between the Australian and New Zealand fauna and that of Chile [South America]; a relationship exemplified in abnormal forms, apparent survivals of past ages, of which we already have" many other equally remarkable instances in all branches of animal life, recent and fossil. And precisely similar is the relationship between *Tanyderus pictus*, of the same family, from Chile, with twenty-five antennal joints, and *T. ornatissimus* from Amboina, with twenty-two joints.

Gynoplistia, another tipulid genus, from Australia, New Zealand, New Guinea and Celebes, with numerous species, has from sixteen to twenty antennal joints, branched like those of *Cerozodia.* Are these forms really survivals of primitive types? I do not think that we are permitted to doubt it. Their habitats and distribution alone indicate that, and the fact that three of the few known forms of diptera with multiarticulate antennæ are known only from the Miocene is also corroborative. *Magachile*, perhaps identical with *Protoplasa*, is one of these amber forms.

Among the Cecidomyidæ we have a few forms with multiarticulate antennae, as many as thirty-six in the males of some *Hormomyiæ* with a maximum of twenty-four in the females. The only other forms with abnormal multiarticulate antennæ that I can discover in the literature, are *Rhachicerus*, with from twenty to thirty joints, *Chrysthemis*, an amber genus with twenty-three joints; and *Electra*, also from the amber, with thirteen joints, all belonging to the xylophagid "Brachycera," having a normal maximum number of ten joints. The fact that some of these examples have a larger number of joints in the male antenna than in the female, may seem to indicate that the increased number is an acquired secondary sexual character, and that the female antenna is nearer the primitive number. But, why may we not assume that the diminished number in the female is the real acquired sexual character, and not the increased number in the male? Certainly this *must* be the case with such forms as *Tanypus* and its allies among the Chironomidæ, *Micromyza* and others of the Cecidomyidæ. From the frequent occurrence of sexual variation in these apparently primitive forms, I think it is probable that the early diptera all had fewer antennal joints in the females than in the males. I am confident that we are safe in accepting at least thirty-nine as the original and primitive antennal number among diptera; safe in the belief that the evolution of the dipterous antenna has always been by reduction from this primitive number, and never by the reacquirement of joints once lost.

Just how the loss of antennal joints has occurred is not always clear. It may be assumed that it has been by the loss of distal segments, but this is certainly not always the case, especially when these distal joints have been highly specialized. The scape is perhaps never entirely reduced. The genus *Chionea*, a wingless tipulid, has the conical third antennal joint terminating in a slender, three-jointed style, a structure very much like that of the Nemestrinidæ, for instance. Do these joints represent the first four of the flagellum? Osten Sacken thinks that the reduced number of twelve joints in *Toxorhina*, belonging in a group having the normal number of sixteen, is due to the coalescence of the basal joints of the flagellum. The stratiomyid genus *Chrysochlora*, as one of numerous instances, with the normal number of flagellar joints, has the last or eighth specialized into a slender arista. Is this arista homologous with the arista of the housefly, for instance, where it is serially the fifth or sixth? The defect of the Comstock-Needham system of venation nomenclature is the assumption that the disappearance of veins has always been due to coalescence, whereas we positively know that in many cases it has been due to their loss without coalescence. Has the reduction of the flagellum been the result of the close fusion of segments, or the absolute loss of proximal ones; or has the arista been variously and repeatedly produced by the attenuation of the last segment or segments, whichever they happen to be? I believe that the arista has usually resulted from the former method and that it generally is homologous. It is quite clear, however, that the diminution in the number of homologous or homonymous joints has often been due to the loss of distal segments, whatever may have been the case with heteronymous forms; and the proof of this is apparent in the oftentimes vestigial condition of the terminal joints. The subject, however, is one worthy of investigation, and may throw light on the relationships of many of the diptera.

Sixteen antennal joints seem to be the primitive normal number of the modern Nemocera, a number acquired so long ago that very few examples yet remain of the more primitive condition. Do they indicate a single phylum? It seems doubtful. The forms with multiarticulate antennæ not only belong in the three chief subdivisions of the Tipulidæ, but are also found among the Cecidomyidæ, which would seem to indicate that the number sixteen had been acquired independently in different lines of descent — that family or subfamily differentiation had occurred before the final reduction took place. It is also curious to observe that the minimum number of antennal joints in the Tipulidæ, Cecidomyidæ and Chiromomidæ is six, precisely the maximum number of the Cyclorrhapha.

In living forms the maximum and very common number of flagellar joints among the Brachycera, with the exception of *Rhachicerus*, is eight, found so frequently in the Xylophagidæ, Stratiomyidæ, Tabanidæ and Acanthomeridæ. Is this coincidence of phylogenetic significance? I feel quite sure that it is. I think that no one can dispute the relationships between *Rhachicerus* and the Xylophaginæ. Is *Rhachicerus* a belated survival of the xylophagid ancestors? If so, the phylum must have branched off long ago from the Tipulidæ (the venation excludes all other families, save the Rhyphidæ), before the antennæ had become reduced below thirty segments. One thing at least seems very evident, the Rhyphidæ are not the nearest related to the Xylophagidæ of the nematocerous families, as is usually believed. The Brachycera had their origin evidently directly from the ancestral Tipulidæ.

Likewise all of the five-jointed families would seem to be excluded from ancestral relationship with the six-jointed forms, and especially the Cyclorrhapha, though possibly the reduction has occurred since divergence.

While the antennæ, taken separately, may offer suggestions as to phylogenies of the dipterous families, and while they may absolutely veto such theories as imply reversion, they can settle none by themselves; they must be correlated with all the other organs of the body, and must harmonize with theories derived from other organs. I offer, nevertheless, the foregoing suggestions, in the possibility or probability that they may find corroboration

Secondary sexual characters are transmitted by heredity to the other sex, unless inhibited by sexual utility, or, possibly, sexual selection. It was for the casual statement of this law to a class in paleontology that I have recently been made the victim of a sensational press. The primitive eyes of diptera were doubtless separated by the front equally in both sexes. As a sexual character the eyes have become contiguous above the antennæ, almost invariably a male character. There is evidently a sexual use for this greater development of the eyes in the male that has preserved the character with but little tendency to transmission to the female. It has, however, been transmitted to the female in some instances, and there are a very few forms in which the female has acquired the character in advance of the male. Examples of the former may be found among the Cyrtidæ, but a better one is that of *Systropus* of the Bombyliidæ, with contiguous eyes in both sexes, while the very nearly related *Dolichomyia* has the female eyes separated by the front. It was for the contiguity of the eyes in the male that Osten Sacken twenty-five years ago proposed the convenient term " holoptic," the antithesis of which, " dichoptic," was suggested by me a little later. But Osten Sacken's meaning of the term has been somewhat misunderstood. He gives as a definition of his Nemocera vera the nonholopticism of the eyes, while it is well known that some forms in this group do have contiguous eyes. But Osten Sacken really meant sexual holopticism, not simply contiguity of the eyes on the front. It remains to be proven that sexual holopticism does not really occur among these families of flies. If so, however, the occurrence must be extremely rare.

The primitive dipteron must have had eight fully developed longitudinal veins (including the auxiliary vein), with the second, third, fourth and fifth furcate; and a complete discal cell. The head was rather small, with the compound eyes separated equally by the front in both sexes. The ocelli were functional, and the maxillary palpi had four freely articulated joints ; the labial palpi had probably already disappeared, though Wesche thinks differently. There were at least thirty-nine antennal joints in the male. The prothorax, mesothorax and metathorax were imperfectly fused, and the metanotum was visible from above. The abdomen had nine functional segments ; the body was without differentiated bristles ; and the tarsi had membranous pulvilli and empodia. The primitive flies were of moderate or small size, and probably crepuscular in habit, or at least denizens of shady forests.

Of modern diptera the Tipulidæ approach most nearly this hypothetical ancestor, but they have become specialized by a general increase in size, by the almost complete loss of the ocelli ; by the loss of the pulvilli ; and the loss of the branch of the third vein, save among some of the Ptychopterinæ. The Rhyphidæ come next, but they have acquired holoptic eyes in some forms. Of the other families, the Psychodidæ and Culicidæ are perhaps the nearest allied to the original type, notwithstanding the occasional occurrence of multiarticulate antennæ among the Cecidomyidæ. The Culicidæ evidently represent an old type geologically, recrudescent in later times. Their fixed venation and antennal structure could only have come from long inheritance in a phylum which has not yet reached decadence. The blood-sucking habit of the mosquitoes is doubtless a rather recently acquired one, probably since the great development of the warm-blooded animals, as is evidenced by the almost innumerable sexual modifications of the palpi, modifications seldom found among the other families of Nemocera. The mosquitoes doubtless arose from the Corethrinæ, now decidedly on the wane. Every family, save the Tipulidæ, is I believe, absolutely excluded from immediate genetic relations with the Brachycera, because of the venation and the antennæ. I am, upon the whole, inclined to the belief that Osten Sacken was right in insisting upon the taxonomic importance of the Nemocera, as one of the chief phyla of diptera.

DO ANTS FORM PRACTICAL JUDGMENTS?

C. H. TURNER.

Scattered through the literature are several records of observations which indicate that ants form what Hobhouse calls practical judgments. However, recent comparative psychologists favor the casting of such evidence out of court, because it was not obtained from experiments conducted under proper conditions of control. Lubbock[1] experimented upon the subject with negative results. The first two bits of evidence reported in this communication are reports of mere observations; the only excuses for recording them are that the observations were made in the laboratory and that they confirm the records of similar observations made by others in the field; the third, however, is the result of a carefully planned and controlled series of experiments.

For several months I have had in one of the laboratories of the University of Chicago a colony of *Camponotus herculeano-ligniperdus*, consisting of nine winged females and about twice as many workers.[2] The Janet nest in which the ants were housed contained a row of three compartments (Figs. 1 and 2; *A*, *B*, *C*). The entrance to this nest (Fig. 1, *E*) was about one centimeter wide by two centimeters high. For several weeks no obstructions of any sort were placed by the ants in that entrance, although a large amount of litter was kept upon the island at all times. An ant usually mounted guard in the entrance *E* and similar guards were usually stationed in the tunnels connecting compartment *C* with compartment *B* and compartment *B* with compartment *A*. For over two months I examined the nest several times daily and in over ninety per cent. of the times I found guards located in the places mentioned.

In the course of some experiments upon an entirely different problem I had occasion to fight the guard stationed in the entrance

---

[1] Lubbock, "Ants, Bees and Wasps," London.

[2] To prevent the experiments recorded in this paper from being invalidated by mice, all holes leading into the room were filled with plaster of paris and the door was kept locked whenever I was absent.

E (Fig. 1) with dissecting needles and glass rods. Sometimes I used plain needles or glass rods, at others needles or rods that had been moistened with oil of cedar or oil of cloves. Each time the fight was continued until the ant retreated into the innermost part of the nest. After these maneuvers had been repeated several times daily for about a week, the guard withdrew from the entrance *E* and the ants plugged that passage-way with detritus, composed of bits of wood and bread from the island, trash from the interior of the nest and cotton stripped from the turkish toweling of the nest. For five months thereafter the nest was examined from five to ten times every day, but no ant was observed as a guard, in either entrance *E* or the tunnels connecting the apartments until August 10, 1907. Since then they have been mounting guard as regularly as they did before they plugged the entrance.

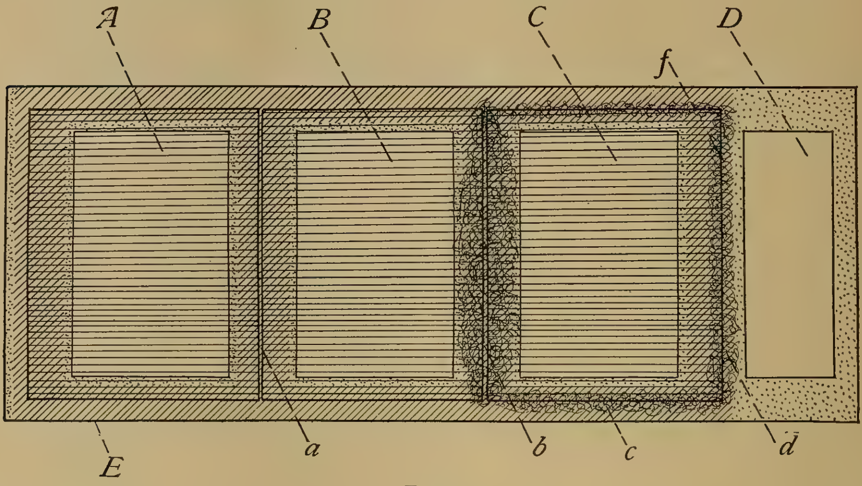


Fig. 1.

On the same Lubbock island with the above-mentioned *Camponotus-herculeano-ligniperdus*, I had a large colony of *Formica fusca* var. *subsericea* Say. The island was kept littered with detritus of various kinds. One day I noticed a worker of this colony begin the construction of a bridge across the ditch of water that surrounded the island on which the colony was located. This partial bridge was constructed in the following manner. The ant placed a piece of charred paper about one centimeter wide upon the water on the inner side of the ditch. After walking out upon this bit of paper and reaching outward with its antennæ, the ant returned to the island and picked up a crumb of bread crust three millimeters wide. The ant then walked across the charred paper and placed this bit of bread upon the water just beyond and adjacent to the paper. After standing for a short time upon the outer edge of the crumb and reaching outward with its antennæ, the ant returned to the island and picked up a piece of wood two millimeters wide by three millimeters long. This the ant placed upon the water just beyond and adjacent to the crumb. Thus there was constructed, extending three fourths of the distance across the ditch, a bridge of three elements. Upon the outer terminus of this partial bridge the ant stood for fully two minutes, reaching continually outward with its antennæ. The bridge was never completed.

The third bit of evidence was afforded by a series of experiments performed upon the same colony of *Formica fusca*. This colony, consisting of three wingless perfect females and about two hundred workers, was brought into the laboratory September 27, 1906. It was from the sidewalk of one of the streets of Chicago, where it was located partly beneath the stone and partly in the trash that the ants had heaped up along the edge of the stone. The sidewalk, which was several years old, was composed of concrete stones at least a yard square and fully six inches thick. The stones of this particular pavement were free from cracks of all kinds. In the laboratory the ants were kept in a nest of the type described above. Compartments *C* and *B* were covered with orange glass and compartment *A* with colorless glass. Throughout all of the experiments the Lubbock island upon which the nest was located was kept littered with bread crumbs, bits of wood, small pieces of egg shell, partially burned matches, charred paper, cotton, etc. For nearly three months the ants of the colony were the subjects of daily experiments upon the sense of hearing, etc. After that, for several weeks, the ants were left to themselves, but the nest was carefully scrutinized several times every day. At all times some workers would be found in apartment *B*, but the fertile females and the majority of the workers used compartment *C* (Fig. 1) as a living room. Whatever booty the ants captured was always carried into this chamber.

On the twenty-sixth of December, 1906, I discovered three batches of newly laid eggs in the living chamber. For several days prior to this discovery, an ant from the nest had been busy covering crack *b* (Fig. 1.) with detritus obtained from the island. At first only one ant was thus occupied. Later in the day a second ant joined this one. On some days three and on others four ants were thus engaged. These ants worked on for about two weeks and covered not only crack *b* but also the edges, *d*, *c*, *f* (Fig. 1), in the order named. Crack *b* received the largest amount of trash, edge *d* the next largest amount, while the edges *c* and *f* each received about an equal amount. The ants covering these cracks sometimes obtained the trash from one place on the island and sometimes from another; thus all the trips of the same ant were not made along the same path. Not only so, but the same ant often went to the trash pile along one path and returned to the crack along another.

In this particular experiment the glass cover over *C* reached much nearer the edge of the well than is shown in the illustration, which was drawn from another experiment of the same kind. Indeed, it entirely covered the turkish toweling on the well side of the living chamber. As a result of this, when the ants began to cover the edge *d* with trash, it would fall down into the well. This continued for nearly three days and by that time the well contained quite a collection of bread crumbs, bits of wood, and the charred ends of matches. About the close of the third day, the ants stopped carrying heavier debris and began covering the edge *d* with fibers of cotton shredded from the layer of cotton upon which the nest rested. They continued to add cotton fibers for about a day, at the end of which time they recommenced adding wood and bread crumbs to the pile. This time, owing to the presence of the cotton fibers this coarser detritus remained where placed. I do not feel justified in attaching much significance to the fact that after the other detritus failed to remain on the crack the ants covered it with cotton fibers and then resumed carrying heavier materials; for the cotton was all brought from a side of the nest upon which there was no other detritus and it may have been that the ants happened to go to that side for detritus and, finding cotton in abundance, continued to return to that side for material.

While these one to four workers were busy covering the cracks surrounding compartment *C*, yet others were busy filling compartment *A* (Fig. 1) with trash. A large number of workers assisted in filling compartment *A*, hence it was not long before this compartment was almost completely filled with trash and the entrance *E* so reduced in size that it was necessary to enlarge the opening whenever occasion arose to carry large pieces of captured food to those within the nest.

On an adjacent island I had a colony of the same species of ants in which there were no fertile females. In the early part of May these neuters began to lay eggs. Immediately compartment *A* was filled with trash.

Since the glass covering compartment *A* is colorless we must look upon the detritus placed there as trash heaped about the entrance to the nest. That the formation of such a trash pile about the opening of the nest is a common breeding habit or instinct is evidenced by the fact that in nature trash piles are found about the openings of many of the nests of this species. In the early spring I have frequently noticed such trash piles in the process of formation. They are composed partly of dirt brought from within the nest and partly of trash gathered from the surrounding territory. In a region where this species of ants is common, a careful search in the early spring is certain to reveal several such trash piles in the process of construction. In almost every case observed by me, a few ants were busy collecting trash from the outside and dropping it about the nest opening, while a larger number of ants were bringing dirt from the interior and heaping it about the same opening. It would then be illogical to consider the trash stored away in compartment *A* as anything more than the homologue of the trash pile that this species frequently builds about the entrance to its normal nests.

To me it does not seem logical to group the four piles of trash covering crack *b* and the edges *c*, *d*, *f* (Fig. 1) in the same category, for neither of these trash piles surrounded an entrance into the nest: *b*, the nearest of these piles, was at least seven inches from *E*, which was the only opening into the nest. Since this colony and its immediate ancestors had lived for several generations under the paving stones mentioned it is unlikely that either they or their immediate ancestors had ever experienced a nest with a crack leading into the brood chamber.

To make sure that the covering of the cracks mentioned above was not a mere coincidence, I removed gently the trash that was covering crack *b*. In less than an hour a few ants were busy covering it. At intervals of about a week, this experiment was repeated twelve times; always yielding the same results. Usually one or two ants did the covering; at no one time have I seen more than four thus occupied.

That the ants were bent not on covering just any cracks that entered the nest, but only the cracks that affected the brood chamber is evidenced by the fact that, although these experiments covered a period of eight months, at no time did the ants cover the crack *a* or the free edges of the glass covers of compartments *A* and *B* (Fig. 1). Furthermore ants bearing trash would frequently cross the edges of the covers to *A* and *B* and even the crack *a* and pass on and deposit their burdens on the crack *b* or the edges *c*, *d*, or *f*. There is yet another evidence of this statement.

If this behavior is for the purpose of covering cracks the existence of which alters the conditions in the brood chamber, a crack crossing the brood chamber should produce a greater disturbance and hence should be covered by the ants first. Therefore I divided the cover to the living chamber *C* into two equal parts which were so adjusted as to leave between them a transverse crack *e* (Fig. 2) wide enough to make quite an opening yet too narrow to permit the passage of ants to and fro. Whenever this was done, and it was repeated over a dozen times, the first crack to be covered was *e*, and after *e*, *b* (Fig. 2). In each case only a few ants covered the cracks. Usually only one or two were thus employed; at no one time were over six thus occupied. Remember that that nest contained at least two hundred workers.

Whenever the trash was removed from crack *e* (Fig. 2), were it done ever so gently, the induced restlessness of the ants within the nest indicated that they were much disturbed. If I gently breathed against the uncovered crack, the ants within rushed about in all directions as though panic stricken. To one who has watched this ant (*Formica fusca* var. *subsericea*) when outside the nest continue its work even when a breeze was blowing, the pronounced agitation caused by such a slight draught is sure to appear striking. It is, however, in harmony with observations, recorded in a former paper, upon the effect of sound and light upon ants. It seems that the same lights and sounds and draughts which scarcely call forth any response when encountered without the nest, induce quite vigorous responses when encountered within the nest. In other words how an ant responds to a certain stimulus often depends upon whether the ant is within or without the nest.

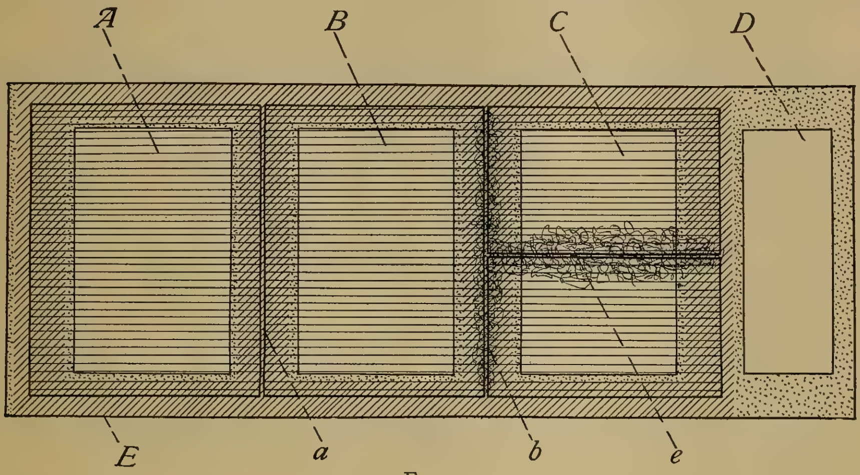


FIG. 2.

What is the cause of this crack-covering behavior? Three possible solutions suggest themselves: first, the covering of the crack is a reflex activity induced by draughts through the cracks affecting the ants within the nest; second, it is a reflex response caused by odors that emerge through the crack and stimulate passing ants to cover it with trash; third, it is a response due to certain ants grasping the fact that the crack needed to be closed and then proceeding to cover it. The last supposition predicates to them what Hobhouse would call a practical judgment.

If the first assumption be true, then all of the ants within the brood chamber should have been stimulated to do the same thing. Such was not the case; for although there were about two hundred workers within the nest, never more than six were seen covering the crack at any one time. Sometimes it took the few ants employed several days to cover the cracks in a satisfactory manner; yet in no case did a larger number participate.

To test the second assumption two different kinds of experiments were performed. I shall first describe an experiment of the first kind. When all the ants were resting quietly within the nest the trash was gently removed from crack *e* (Fig. 2). The ants within the nest immediately became restless. Two of them mounted the ceiling and examined the crack carefully. About fifteen minutes later an ant was observed moving back and forth on top of the nest. On coming in contact with crack *e* it paused momentarily, examined the crack carefully and then passed on. After it had roamed about the island for a few minutes, I imprisoned it. About five minutes later another ant from the nest walked across the top of the nest to crack *e*. After examining the crack carefully with its antennæ, the ant began to cover the crack with trash. In a few minutes this ant was joined by a third. This experiment shows that all ants that cross the crack are not stimulated to do the same thing.

The second type of experiment was quite unlike this. As before, the trash was gently removed from crack *e* producing the same restlessness within. The cover was then removed from compartment *B* (Fig. 1) and several worker ants transferred to a beaker. The cover was then replaced on *B*.

One of the captured ants, after having been marked with a characteristic color was placed near the middle of the top of compartment *C* and covered with a transparent white glass cone eight centimeters in diameter. At first the ant would make vigorous efforts to escape from the cone. It would meander over the top of the nest, go repeatedly round and round the circumference of the base of the cone and sometimes mount its sides. In these random movements the ant would of necessity cross that crack many times. After more or less of this fruitless activity, the ant would become quieter. It would then move more leisurely about, examining the crack at frequent intervals or else it would rest over the crack or a short distance therefrom and quietly preen its antennæ. When this condition of calm had been attained, which usually required about five minutes, the glass cone was quietly removed. Thus the confining cone was not removed until the ant had recovered from the excitement caused by the handling.[1] Now if contact with crack *e* or the odors ascending through it will induce ants reflexly to cover it with trash, then, when free to roam at large, the ant should have proceeded to cover the crack with trash. This was tried with twelve different ants, but in no case did the ant cover the crack with trash. In all cases the ant carefully examined the crack and in one case it tried to force its way through the crack into the nest. Sooner or later the ant would begin to roam about over the top of the nest and in some cases over the Lubbock island as well. After awhile it would enter the nest. Some ants entered the nest within a minute after the confining cone was removed, one spent two hours finding its way home, one became lost and the majority took less than three minutes to find the entrance to the nest. About an hour after the beginning of the experiment an unmarked worker from the nest began to cover the crack with trash, and by the close of the third day it had covered cracks *e* and *b* in the usual manner (Fig. 2).

Once or twice a week for several months I continued to remove the trash from crack *e*, soon after it had been completely covered. Each time the crack was recovered. On June 28, however, the ants not only covered the top of *e* with trash, but beneath *e*, on the inside, they built up a wall of detritus, through which a tunnel connected the two halves of compartment *C*. The outside cover was composed of a heterogeneous mass of coarse particles of various kinds and a few cotton fibers; the partition constructed on the inside consisted of a felted mass of cotton fibers and fine crumbs of bread. Nine times I destroyed this inner portion; each time it was reconstructed by the ants out of the same kind of material. The tunnel through the partition was sometimes located in one position and sometimes in another. The different partitions varied in width from one fourth to three fourths of an inch; the tunnels through the partition varied in width from one half an inch to one half the length of the partition. The ants glued the partition to the roof in such a manner that no matter how wide the tunnel, the felted roof always completely closed the crack.

---

[1] Experiments recorded in my paper on "The Homing of Ants" show that the handling of ants to mark them with water colors does not alter their physiological attunement.

After the ants had begun to close the crack with the wall built up within from the floor, the crack *e* was thereafter only imperfectly covered with trash; furthermore, instead of covering the edges *b*, *c*, *d* and *f* with trash, the same result was obtained by chinking from inside the space between the glass cover and the top of the walls of the brood chamber with material similar to that with which they constructed the inner partition.

To see if all colonies of *Formica fusca* var. *subsericea* Say would behave in the same way in the presence of a crack across their brood chamber, a crack was made in the top of the brood chamber of each of four nests of this species. These nests were obtained from the field and housed in Janet nests for this special purpose. In two cases the ants with their young deserted the chamber over which I had placed a crack and migrated to the nest of another colony of the same species.[1] In one case the ants with their young migrated from the compartment over which I had placed a crack into another compartment of the same nest. I forced them back into chamber *C* by substituting a piece of colorless glass for the orange glass with which the chamber into which they had migrated was covered. They and their young remained thereafter in chamber *C* for six weeks without doing anything that tended to close up that crack. In the fourth case the ants with their young retreated from brood chamber *C*, over which I had placed a crack, into chamber *B*. I then placed a crack across chamber *B* and a complete cover over chamber *C*. At once the ants covered the crack with trash but no partition was constructed upon the inside.

It is convenient to group the responses of animals living in colonies into class responses and individualistic responses. A class response is a stereotyped response which would be made by any and each member of a group when confronted with similar stimuli. When, under identical conditions, one member of a group responds to a stimulus in one way and yet other members respond to a similar stimulus in a different manner the response would be individualistic. When an animal faces a situation for which it has no class response and yet almost immediately makes an individualistic response which overcomes the difficulty, it has formed what Hobhouse calls a practical judgment.

---

[1] The colony into which each of these colonies migrated contained, before the arrival of the emigrants, no fertile females.

It seems to me that this is what the ants did in the case recorded here. When a crack was made into their brood chamber they were face to face with a situation for which they had no class response. After awhile one to a few individuals made individualistic responses which resulted in the closing of the crack. To those few ants the disturbance in the brood chamber had been associated with the unclosed crack. To them the crack had acquired a meaning. It had become a crack-to-be-closed and they proceeded to close it.

It is not claimed that the construction of a trash pile of heterogeneous material, nor even the building of a felted partition out of special materials, indicates the formation of a practical judgment; for the forming of a trash pile by ants is, and the modeling of a partition may be, an instinctive action. But the utilization of these instinctive activities, without a preliminary period of experimentation, to meet adequately conditions for which the ants had no stereotyped response is what warrants the assumption that they form practical judgments.

It seems to me that in constructing the partial bridge, in removing the guards from the entrance and plugging it with cotton, and in closing the crack to the brood chamber, at first with trash piled on the outside and later with a wall built up from within, the ants have responded to stimuli, not as ends in themselves, but rather as means to ends. This would lift the act out of the realm of instinctive behavior into that of the practical judgment.

Hull Zoölogical Laboratory,
University of Chicago, August 17, 1907.

Explanation of Figures.

Each represents a diagram of the top of a Janet nest: *A*, *B*, *C* are brood chambers; *D* is the water well; *E* is the entrance to the nest; *a*, *b* are cracks between the glass covers; *c*, *d*, *f* are edges of the glass cover of chamber *C*; *e* is a crack across the top of chamber *C*; the oblique shading represents the turkish toweling; the horizontal shading represents the glass covers.

THE CAUSATION OF MATURATION IN THE EGGS OF LIMPETS BY CHEMICAL MEANS.[1]

JULIAN MAST WOLFSOHN.

In his experiments on the maturation of the eggs of the starfish (*Asterias forbesii*)[2] Dr. Loeb found that the eggs when removed from the ovaries of the animal are in most cases immature, but that if they come in contact with sea water, during the breeding season they begin to maturate. The immature state is characterized by a large, plainly-visible nucleus, which, during maturation, becomes invisible. This process of maturation is completed in from one to two hours after the eggs are removed from the ovaries and placed in sea water. Only when the process of maturation is completed is it possible to fertilize the eggs with sperm.

Experimentation on the maturation of these eggs showed that the chemical conditions necessary to cause or accelerate the maturation processes are, that there must be present in the sea water two substances, free oxygen and hydroxyl ions of a certain concentration. Dr. Loeb found further that if, upon becoming mature the eggs were not caused to develop by the addition of sperm, then they died very rapidly. The change in the appearance of the egg after death is very marked; the living egg having a light yellow color, after death it becomes black; and where in the living egg the protoplasm is homogeneous and somewhat transparent, in the dead egg it becomes granular and opaque. Thus it was found that in a culture that had been standing for twenty-four hours, all the eggs that had remained immature were alive, and all those that had matured were dead. This shows that the mature eggs of the starfish die in the course of a few hours, while under exactly the same conditions the immature eggs remain alive.

When the maturation is prevented artificially through lack of oxygen or by the addition of either acid or potassium cyanide to the sea water the eggs remain alive a considerable period of time. The eggs in which maturation has already begun, or has just been completed, are also saved from rapid death by these means.[1]

---

[1] From the Herzstein Research Laboratory of the University of California.

[2] BIOLOGICAL BULLETIN, Vol. 3, No. 6, Nov., 1902.

Later, he found that the eggs of a mollusc (*Lottia gigantea*) when removed from the ovary were always immature and would remain so even if they were kept in water for two days. Such eggs could not be fertilized by sperm. But if they were first treated with a mixture of 50 c.c. sea water and 1 c.c. 1/10 *n* $NaHO$ for from four to five hours maturation took place with the result that over 75 per cent. of the eggs would develop into larvæ in normal sea water after the addition of sperm. He found also that treatment with sea water containing benzol would cause the eggs to become mature very rapiply.[2]

As in the case of the starfish egg the maturation processes in the eggs of *Lottia*, induced by alkali or benzol can be prevented by lack of oxygen, or by either the addition of acid or potassium cyanide to the sea water.

The fact that the immature eggs of the starfish and especially those of *Lottia* could be caused to become mature in this way, suggested experimentation upon the other limpets found at Pacific Grove. Four varieties of *Acmæa* were used by the writer. *Acmæa patina*, *pelta*, *persona* and *scabra*. The eggs of the *Acmæa* studied are all immature when removed from the ovaries. They look very much like those of *Lottia* (Fig. 1) and are characterized by a greenish color, a very irregular outline, and a transparent membrane — a chorion which conforms to the shape of the egg. In maturing the chorion disappears and the egg becomes perfectly spherical in shape and rather more opaque, Fig. 2. Only when this maturation process is completed is it possible to fertilize the egg by the addition of sperm. The eggs of *Acmæa* remain immature in sea water for two or three days and only occasionally does one find any mature eggs in the culture that has stood for any length of time.

To determine, whether by increasing the alkalinity of the sea water the eggs would become mature, I subjected the eggs to the following solutions: 50 c.c. sea water, plus 0.4 c.c.; 0.7 c.c.; 1.0 c.c.; 1.5 c.c. 1/10 n NaHO for 1, 1.5, 2 and 3 hours. After each period the eggs were transferred to normal sea water and sperm added about fifteen minutes later. At the end of two hours, maturation in most of the cases, was completed. Eighteen hours later the following results were obtained:

---

[1] These experiments have recently been repeated and confirmed by A. P. Matthews, *Am. Journal of Physiology*, 1907.

[2] *Univ. of Cal. Publications*, Nov. 17, 1905, Vol. 3, No. 1, pp. 1–8.

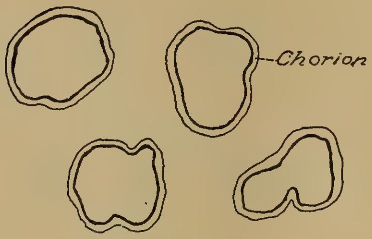


FIG. 1.—Immature eggs.

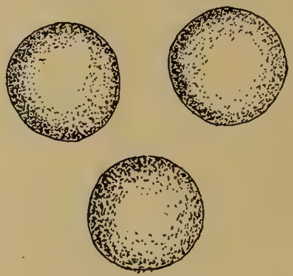


FIG. 2.—Mature eggs.

TABLE I.

Solution.	Length of Treatment with Alkaline Sea Water.			
	1 Hour.	1.5 Hours.	2 Hours.	3 Hours.
50 c.c. sea water + .4 c.c. $n/10$ NaHO.	10 % mature. 1 swimming larva. Some disintegrated eggs.	15 % mature. All swimming.	40 % mature. 10 % swimming. Rest disintegrating.	40 % mature. 5 % swimming. Rest disintegrating.
50 c.c. sea water + .7 c.c. $n/10$ NaHO.	30 % mature. 15 % larvæ. Few disintegrating.	50 % mature. 40 % swimming.	70 % mature. and swimming. 40 % irregular.	90 % mature and swimming. 10 % disintegrating.
50 c.c. sea water + 1 c.c. $n/10$ NaHO.	40 % mature. All swimming.	90 % mature and swimming. Few disintegrating.	90 % swimming. 40 % irregular.	98 % mature and swimming. 10 % disintegrating.
50 c.c. sea water + 1.5 $n/10$ NaHO.	All mature and swimming. Fine larvæ.	98 % mature and swimming. Few disintegrating.	95 % mature and swimming. 20 % irregular. Disintegration.	95 % swimming. 5 % disintegrating.

From the table we see that the greatest number of the eggs become mature and form larvæ when 1.5 c.c. $n/10$ NaHO are added to 50 c.c. sea water, and the eggs subjected to this mixture for one hour. Practically all become mature and the larvæ resulting are excellent.

If, however, the eggs are under-exposed or over-exposed to the solutions they either break up or segment irregularly and it is not very long before these latter eggs too, break up, and disintegrate. The number of disintegrating eggs is smaller when the eggs are treated for one hour with the mixture of 50 c.c. sea water and 1.5 c.c. *n*/10 NaHO than with any of the other solutions used. When sperm was added to the eggs which had not been treated with alkaline sea water, not a single egg became mature or developed into a swimming larva.

Next I tried the effects of lack of oxygen on maturation. The alkaline sea water (50 c.c. sea water + 1.5 c.c. *n*/10 NaHO) was placed in a bottle and connected with the hydrogen generator, and a stream of hydrogen was passed through the solution for two hours. The eggs were then quickly introduced and left for one hour in the solution, with a good stream of hydrogen still passing through. The eggs were then transferred to normal sea water and sperm added. The following table shows the result:

TABLE II.

Solution.	Treatment for 1 hr.
50 c.c. sea water + 1.5 c.c. *n*/10 NaHO with oxygen.	All mature and swimming. Few eggs disintegrating.
50 c.c. sea water + 1.5 cc. *n*/10 NaHO without oxygen.	About half a dozen mature eggs. Two larvæ. No disintegration.

This shows that, as in the case with *Lottia*, the alkali can only cause the maturation of the eggs of *Acmæa* if oxygen be present. If the eggs that had been kept from becoming mature by lack of oxygen were subsequently treated with akaline sea water in the presence of oxygen, practically all became mature, and would, upon the addition of sperm, develop into larvæ.

By stopping the oxidative processes in the eggs through the presence of potassium cyanide, maturation can also be inhibited. Thus, if to alkaline sea water a little potassium cyanide be added no maturation occurs.

Table III.

Solution.	Treatment for 1 hr.
50 c.c. sea water + 1.5 c.c. *n*/10 NaHO.	All mature and swimming. Little disintegration.
50 c.c. sea water + 1.5 c.c. *n*/10 NaHO + 1.2 c.c. 1/10% KCN.	Not one mature egg, in whole culture. No eggs disintegrated.

The processes which were accelerated by the presence of the alkali were completely inhibited by the potassium cyanide and it is especially to be noted that while disintegrated eggs were found in every case in the absence of potassium cyanide, no disintegrated eggs were found when potassium cyanide was present.

If the eggs that had been prevented from becoming mature through the presence of the potassium cyanide, were afterwards treated with alkaline sea water, containing no potassium cyanide, practically all became mature and formed larvæ when sperm was added. This shows further that no permanent injury is done to the eggs by treating them with KCN or by depriving them of oxygen for so short a period of time.

I next tried the effect of fat solvents, such as benzol, chloroform, ether and ethyl acetate, upon the immature eggs to see if by treatment with these substances maturation could be produced, and I found that in every case I got positive results. The method of procedure in general was as follows: 30 c.c. sea water were heated to 45° C. and a known quantity of the solvent was added and the mixture vigorously shaken to ensure complete solution. If the solution is not complete the eggs that come in contact with the droplets of the solute are immediately killed. The solution was then cooled down to 26° C., and the eggs introduced. At definite periods the eggs were removed to normal sea water and sperm added. Eighteen hours after the results obtained in Table IV were noted.

The best results were obtained when the eggs were exposed to the mixture for one minute; 85 per cent. of the eggs developing into larvæ upon the addition of sperm. If the eggs are exposed longer to the solution they become mature, but a large number segment irregularly. These irregularly segmenting eggs finally disintegrate before they reach the larval stage, or in the early larval stage. By this method of treatment the eggs become mature much more rapidly than by the treatment with alkali. Usually from three fourths to one hour after treatment the eggs will be mature, whereas when the eggs are simply treated with the alkaline sea water maturation rarely takes place in less than two hours.

TABLE IV.

Solution.	Treatment for				
	½ Minute.	1 Minute.	1.5 Minutes.	2 Minutes.	2.5 Minutes.
30 c.c. sea water + 2 drops benzol.	25 % mature. 20 % swimming. Good larvae.	90 % mature. 85 % swimming. Good larvae. Little disintegration.	95 % mature. 50 % swimming. More disintegration.	90 % mature. 60 % swimming. Great many disintegrated eggs.	90 % mature. 60 % swimming. 30 % disintegrated.

The maturation induced by the treatment with benzol is also an oxidative process, since it does not occur when the oxidations are prevented in the egg. The addition of 1 c.c. 1/10 per cent potassium cyanide to the sea water inhibits the maturation of the eggs treated with benzol, and it is especially noteworthy that not a trace of disintegration is present in the eggs that were overexposed to the solution in the presence of potassium cyanide. With the other fat solvents I have not as yet been able to get such a large percentage to develop as the table below will show.

TABLE V.

Solution.	Exposure.		
	¾ Minute.	1.5 Minutes.	2.5 Minutes.
30 c.c. sea water + 2 drops chloroform.	10 % swimming.	20 % swimming.	25 % swimming. Many eggs killed.
30 cc. sea water + 4 drops $n/10$ ether.	No larvæ.	5 % swimming.	10 % swimming
30 c.c. sea water + 6 c.c. $n/2$ ethyl acetate.	20 % larvæ.	40 % larvæ.	50 % larvæ.

These last experiments were not worked out as thoroughly as those with benzol on account of the lack of material, but we can readily see that all the solutions tried give positive results.

CONCLUSIONS.

1. As in the case of *Lottia* so also in that of *Acmæa patina, pelta*, *persona* and *scabra*, the addition of a small quantity of alkali to the sea water will cause the eggs to become mature.

2. The presence of oxygen hastens the destruction of the mature unfertilized eggs of *Acmæa*.

3. Lack of oxygen or the inhibition of the oxidative processes in the egg by the addition of a little potassium cyanide to the sea water not only prevents maturation but prevents also the disintegration of the mature unfertilized egg.

4. The treatment of the immature eggs of *Acmæa* with fat solvents, such as benzol, chloroform, ether, and ethyl acetate will also cause them to become mature.

The Heckman Bindery, Inc.
N. Manchester, Ind.
SEP 1958
"Bound-To-Please"

SMITHSONIAN INSTITUTION LIBRARIES
3 9088 01228 0954